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(54) ***LISTERIA* BACTERIOPHAGE P825 AND USES THEREOF**

(75) Inventors: **Holger Grallert**, Weilheim (DE); **Julia Lorenz**, Regensburg (DE); **Anna Scherzinger**, Bernried (DE)

(73) Assignee: **DSM IP ASSETS B.V.**, Heerlen (NL)

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CPC ***C12N 7/00*** (2013.01); ***A01N 63/00*** (2013.01); ***A61K 35/76*** (2013.01); ***C07K 14/005*** (2013.01); ***C07K 16/40*** (2013.01); ***C12N 9/2462*** (2013.01); ***C12Q 1/18*** (2013.01); ***G01N 33/56911*** (2013.01); *C12N 2795/00021* (2013.01); *C12N 2795/00022* (2013.01); *C12N 2795/00031* (2013.01); *C12N 2795/00032* (2013.01); *C12N 2795/00051* (2013.01); *G01N 2333/32* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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Primary Examiner — Nicole Kinsey White
(74) *Attorney, Agent, or Firm* — McBee Moore Woodward & Vanik IP, LLC

(57) **ABSTRACT**

The present invention relates to a novel *Listeria* bacteriophage designated ProCC P825. In particular, the present invention relates to the endolysin PlyP825 encoded by the novel phage ProCC P825 and uses of the novel endolysin PlyP825 for controlling *Listeria* contamination and infection.

14 Claims, 8 Drawing Sheets

Figure 1

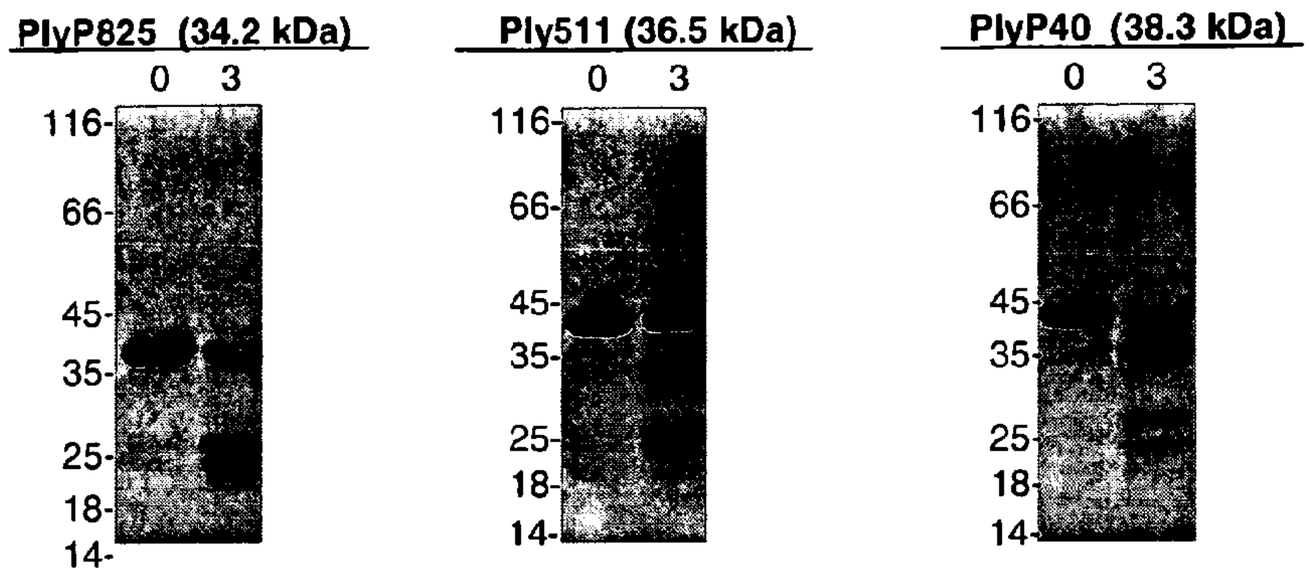

PlyP825 (34.2 kDa)
0 3
116
66
45
35
25
18
14
Ply511 (36.5 kDa)
0 3
116
66
45
35
25
18
14
PlyP40 (38.3 kDa)
0 3
116
66
45
35
25
18
14

Figure 2

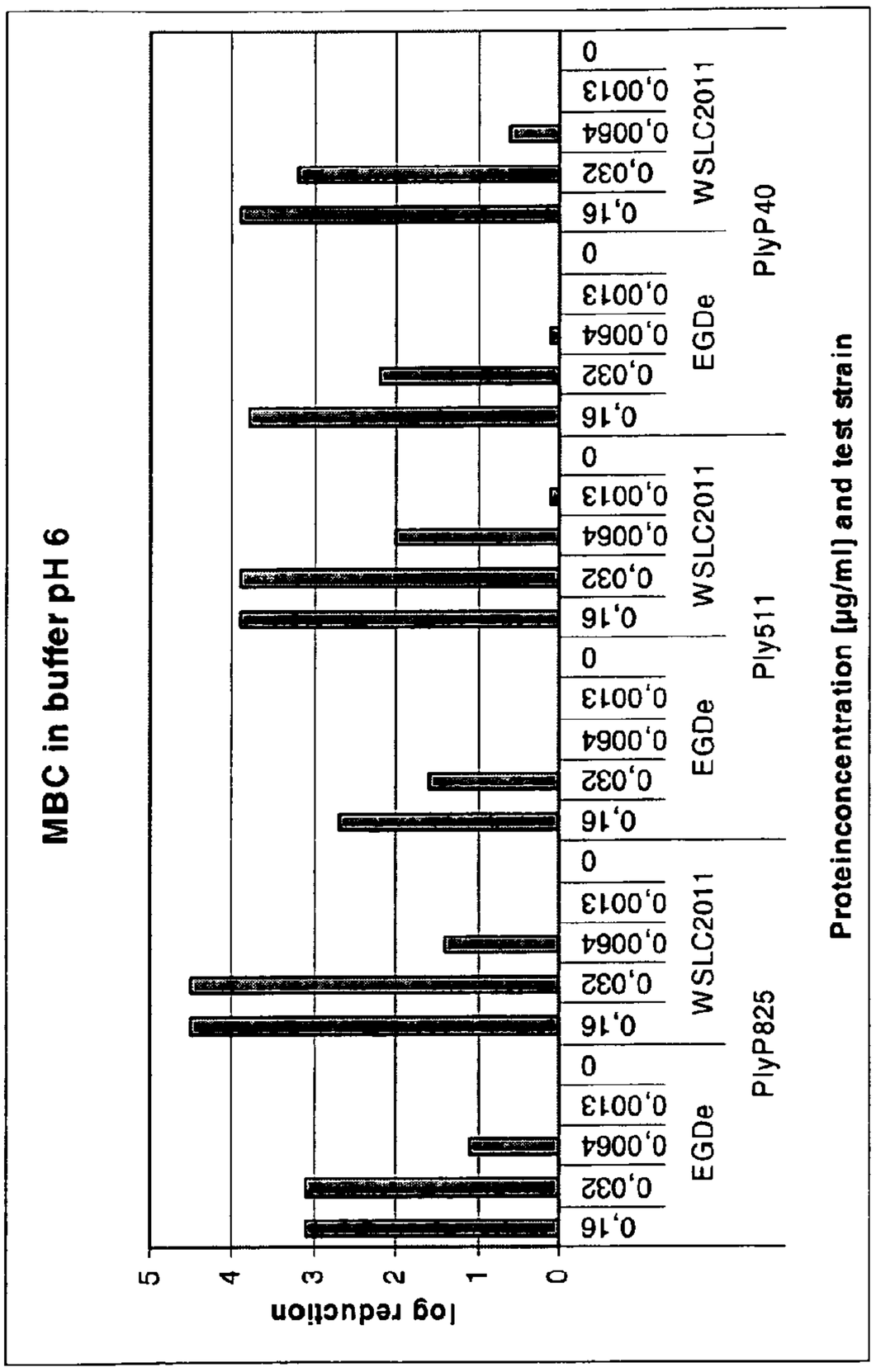

MBC in buffer pH 6
log reduction
0
1
2
3
4
5
Proteinconcentration [µg/ml] and test strain
PlyP825
PlyP82
Ply511
PlyP40
WSLC2011
EGDe
0,16
0,032
0,0064
0,0013
0

Figure 3

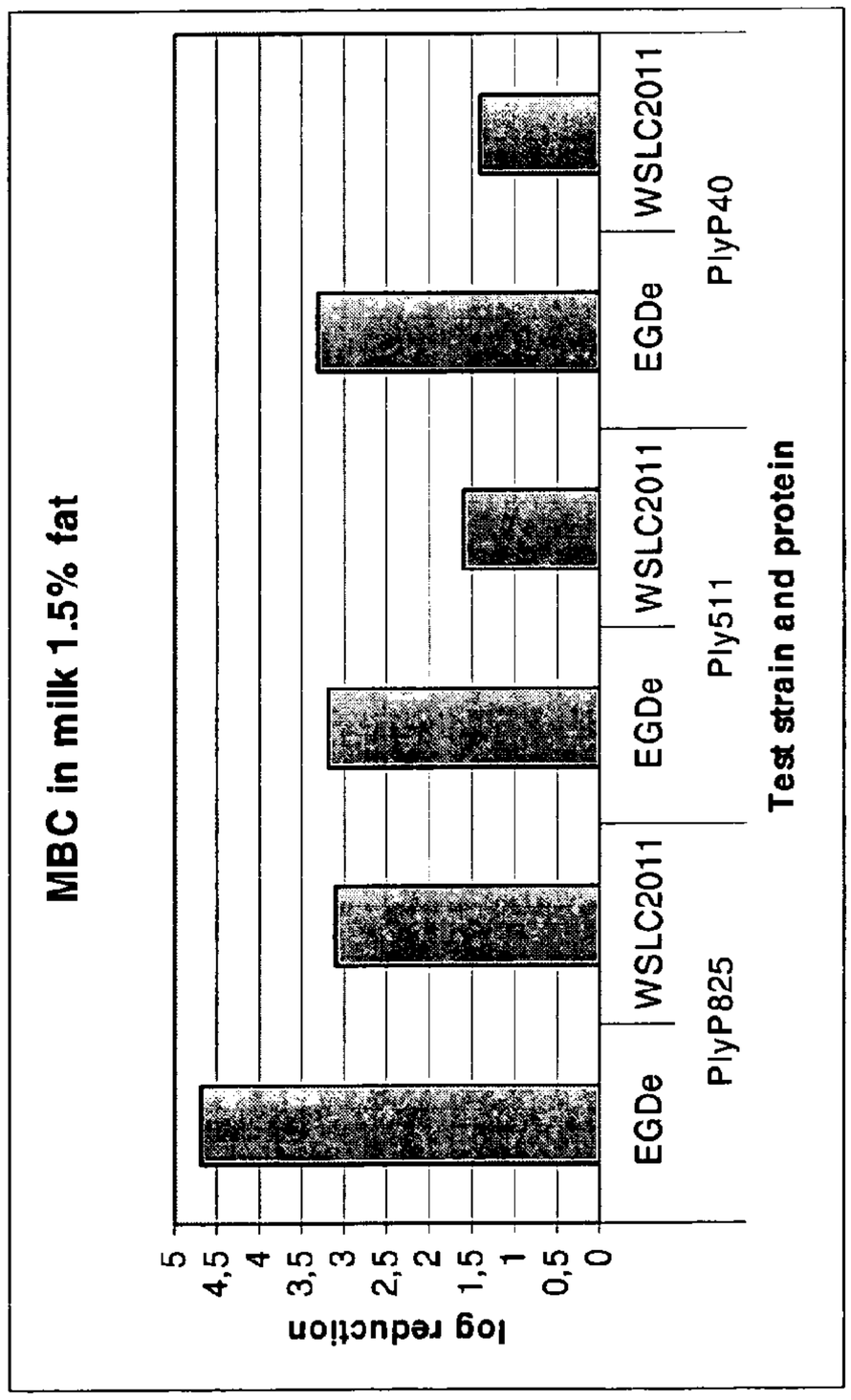

MBC in milk 1.5% fat
log reduction
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
EGDe
WSLC2011
PlyP825
EGDe
WSLC2011
Ply511
EGDe
WSLC2011
PlyP40
Test strain and protein

Figure 4

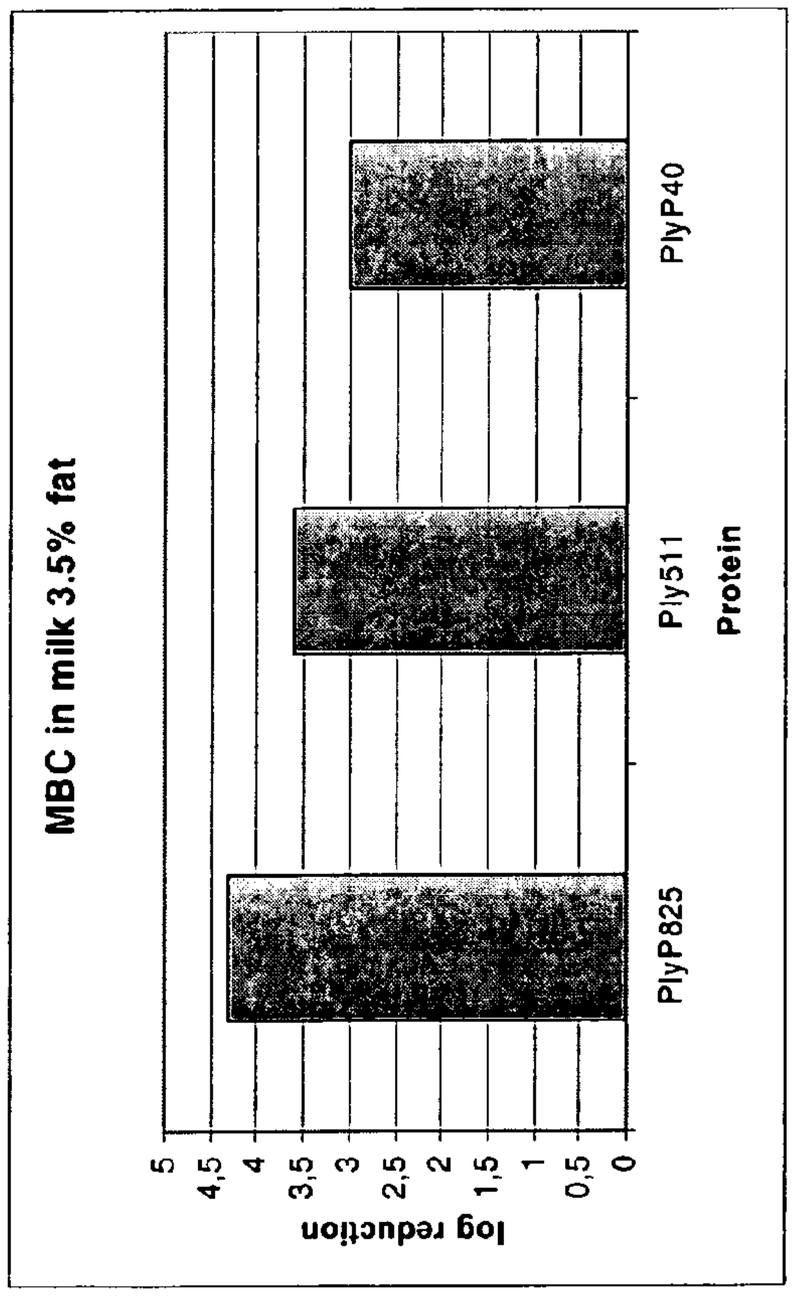

MBC in milk 3.5% fat
log reduction
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
PlyP825
Ply511
PlyP40
Protein

Figure 5

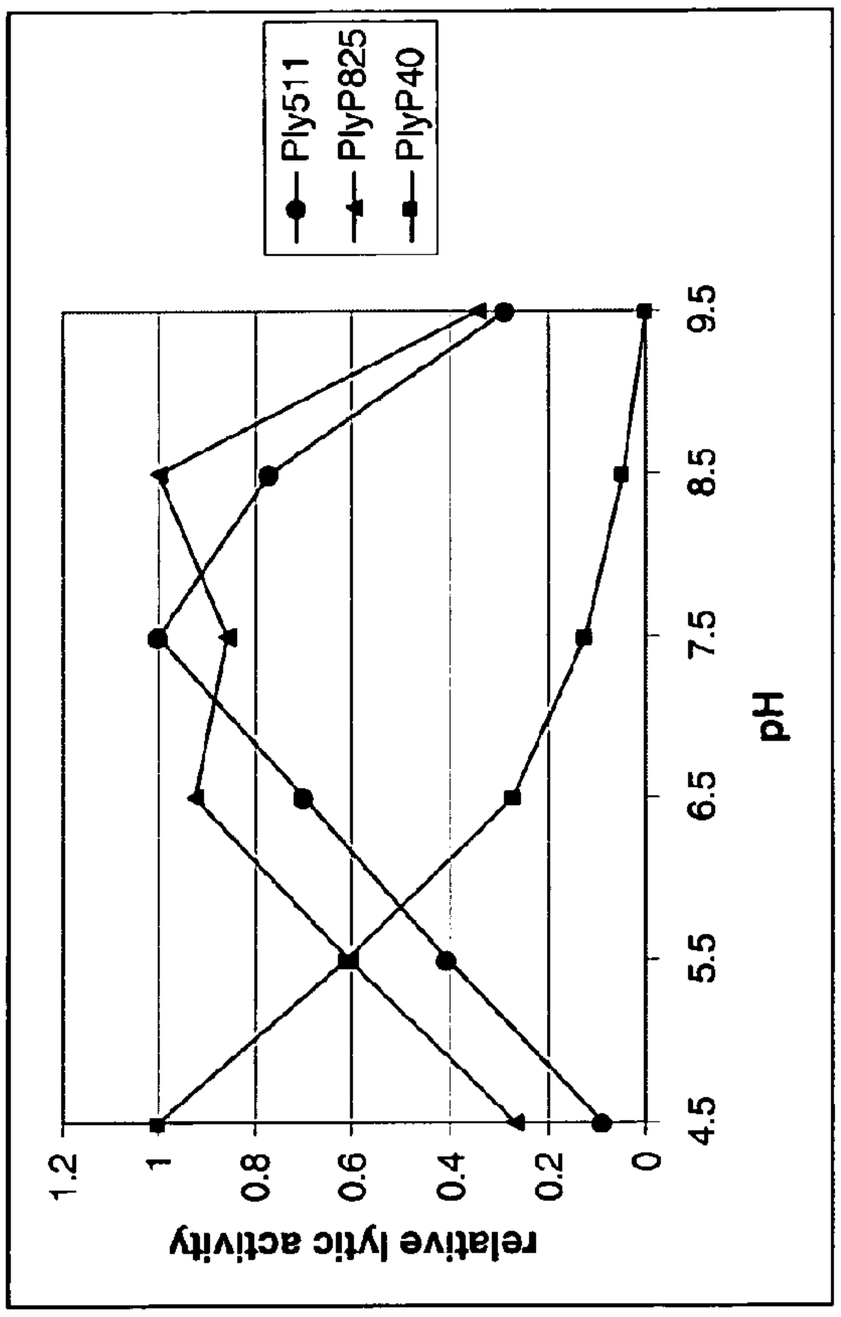

Ply511
PlyP825
PlyP40
relative lytic activity
1.2
1
0.8
0.6
0.4
0.2
0
4.5
5.5
6.5
7.5
8.5
9.5
pH

Figure 6

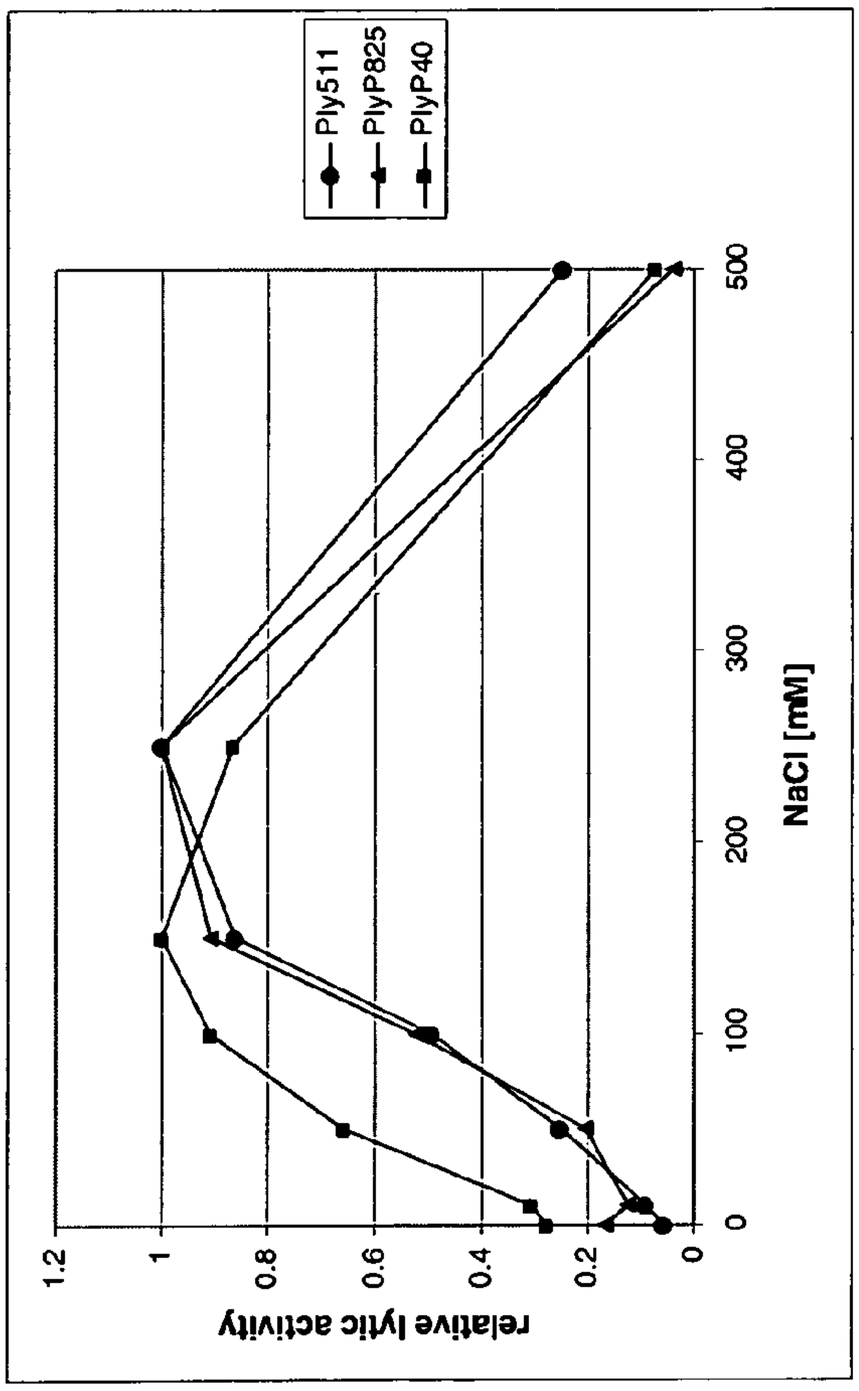
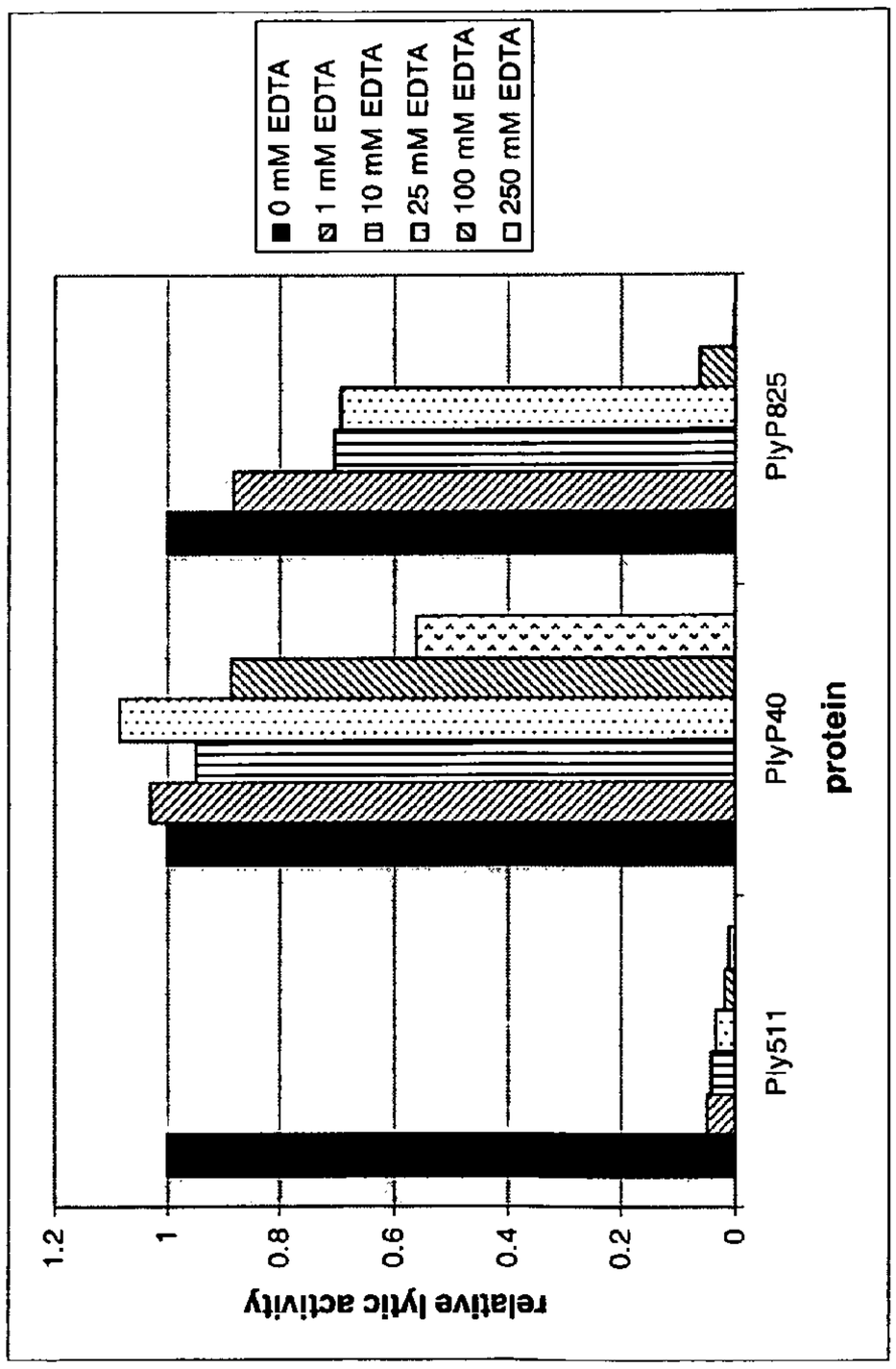

Ply511
PlyP825
PlyP40
relative lytic activity
0
0.2
0.4
0.6
0.8
1
1.2
NaCl [mM]
0
100
200
300
400
500

Figure 7


0 mM EDTA
1 mM EDTA
10 mM EDTA
25 mM EDTA
100 mM EDTA
250 mM EDTA
relative lytic activity
1.2
1
0.8
0.6
0.4
0.2
0
Ply511
PlyP40
PlyP825
protein

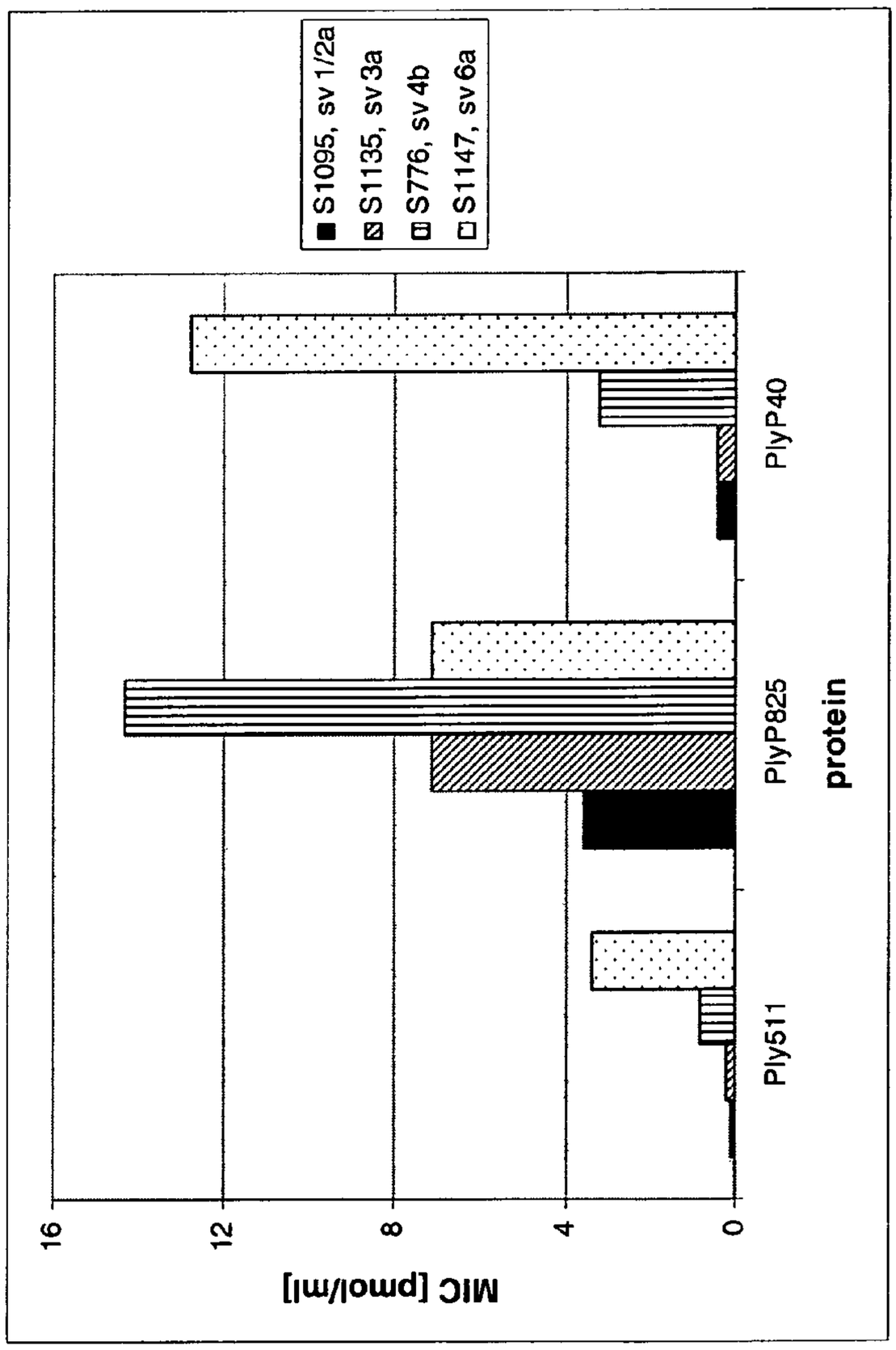

S1095, sv 1/2a
S1135, sv 3a
S776, sv 4b
S1147, sv 6a
PlyP40
PlyP825
Ply511
protein
16
12
8
4
0
MIC [pmol/ml]

Figure 8

LISTERIA BACTERIOPHAGE P825 AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a §371 National Stage Application of PCT/EP2012/002270, filed May 29, 2012, which claims priority to European Application No. 11004348.6, filed May 26, 2011.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a novel *Listeria* bacteriophage designated ProCC P825. In particular, the present invention relates to the endolysin PlyP825 encoded by the novel phage ProCC P825 and uses of the novel endolysin PlyP825 for controlling *Listeria* contamination and infection.

Description of Related Art

The gram-positive bacterium *Listeria monocytogenes* is a bacterial pathogen which is known as the causative organism in several outbreaks of food-borne disease. Listeriosis is a life-threatening infection of humans worldwide which is caused by *Listeria monocytogenes* and which is characterized by a variety of symptoms and conditions, including diarrhea, abortion and encephalitis. In industrialized countries, high mortality is associated with listeriosis following *Listeria monocytogenes* food contamination. In humans, the prevalence of listeriosis has risen significantly since the 1980s, resulting in intensified surveillance of *Listeria monocytogenes* in food industry. This contributed to a decrease of human listeriosis cases in the last two decades (McLauchlin 1987, Oevermann et al. 2008). However, its prevalence has again increased in the last few years (Gillespie et al. 2006, Goulet et al. 2008, Gillespie et al. 2009).

The species *Listeria monocytogenes* encompasses numerous strains and the genetic diversity amongst them is high (Doumith et al. 2004). Various strains have been implicated in both human and animal disease, and current surveillance schemes for foods are based on the assumption that all *Listeria monocytogenes* isolates are potentially pathogenic, resulting in costly recalls in food industry (Oevermann et al. 2010).

While listeriosis is greatly aided by early administration of antibiotics with rapid bactericidal activity against *Listeria monocytogenes*, research to improve food safety is directed to exploring novel technologies such as the use of bacteriophage for specific killing of bacteria.

Bacteriophages are viruses that infect bacteria. They are obligate intracellular parasites and lack their own metabolism. Phages are the natural enemies of bacteria. They are host-specific in that they infect specific bacterial species or even specific strains (Hagens and Loessner 2007). There are a few exceptions like *Listeria* bacteriophage A511, which can infect and kill bacteria within an entire genus. The extreme specificity of phages renders them ideal candidates for applications designed to increase food safety. Phages can be used for biocontrol of bacteria without interfering with the natural microflora.

Endolysins from *Listeria* bacteriophages are promising tools for detection and control of *Listeria* contamination and infection. These proteins have a modular organization, which is characterized by an N-terminal localized enzymatically active domain (EAD), which contributes lytic activity, and a C-terminal localized cell wall binding domain (CBD), which targets the lysin to its substrate.

It is an object of the present invention to provide a novel *Listeria* bacteriophage and novel endolysins against *Listeria*, which exhibit improved properties over known *Listeria* bacteriophages and known endolysins against *Listeria*.

SUMMARY

The present invention provides a novel *Listeria* bacteriophage designated ProCC P825, which has been deposited at DSMZ, Braunschweig, Germany, under international deposit number DSM 23783 in accordance with the Budapest treaty for deposit of cell cultures. In the present invention, the novel bacteriophage "ProCC P825" is simply named "P825". Therefore, whenever reference is made herein to "P825", the novel bacteriophage "ProCC P825" as deposited at DSMZ, Braunschweig, Germany, under deposit number DSM 23783 is meant.

The present invention provides the novel *Listeria* bacteriophage designated ProCC P825 and a novel endolysin designated PlyP825, which is encoded by the novel *Listeria* bacteriophage P825. The novel endolysin designated PlyP825 is encoded by the nucleic acid sequence shown in SEQ ID NO: 1, which comprises 945 nucleotides. The corresponding amino acid sequence of PlyP825 is set forth in SEQ ID NO: 2 and comprises 315 amino acid residues accordingly. The novel endolysin PlyP825 is particularly useful in the control of *Listeria* contamination and infection.

Aspects of the invention are:

1. A bacteriophage capable of lysing *Listeria* serovars 1/2, 3, 4, 5, and 6.

2. The bacteriophage of item 1, wherein the bacteriophage has a genome (i) comprising the DNA sequence of SEQ ID NO: 7; (ii) having at least 90% or 95% sequence identity with the DNA sequence of SEQ ID NO: 7; or (iii) having at least 90% or 95% sequence identity with the DNA sequence of the genome of bacteriophage ProCC P825 deposited under accession No. DSM 23783.

3. A nucleic acid molecule comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2;

(b) a polynucleotide encoding a fragment, analog or functional derivative of a polypeptide encoded by the polynucleotide of (a), wherein said fragment, analog or functional derivative has endolysin activity;

(c) a polynucleotide which is at least 75% identical to the polynucleotide of (a), and which encodes a polypeptide having endolysin activity;

(d) a polynucleotide encoding a polypeptide having an amino acid sequence that is at least 75% identical to the amino acid sequence of SEQ ID NO: 2 and having endolysin activity;

(e) a polynucleotide which hybridizes under stringent conditions to the polynucleotide of any one of (a) to (d);

(f) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;

(g) a polynucleotide which is at least 75% identical to the nucleotide sequence of SEQ ID NO: 1 and which encodes a polypeptide having endolysin activity;

(h) a polynucleotide comprising part of the nucleotide sequence of (f) and which encodes a fragment, analog or functional derivative of the polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein said fragment, analog or functional derivative has endolysin activity; and

(i) a polynucleotide that is the complement of the full length of a polynucleotide of any of (a) to (h).

4. A vector comprising the nucleic acid molecule of item 3.

5. A host cell transformed or transfected with the nucleic acid molecule of item 3, or the vector of item 4.

6. The host cell of item 5, containing a polypeptide expressed from the nucleic acid molecule of item 3, or from the vector of item 4.

7. A method of making a polypeptide encoded by the nucleic acid molecule of item 3, comprising culturing the host cell of item 5 under conditions such that the polypeptide encoded by the nucleic acid molecule of item 3 is expressed, and recovering the polypeptide encoded by said nucleic acid molecule.

8. A polypeptide encoded by the nucleic acid molecule of item 3, or obtainable by the method of item 7.

9. An endolysin protein obtainable from (i) the bacteriophage of item 1 or 2, or (ii) bacteriophage ProCC P825 deposited under accession No. DSM 23783, or a fragment, analog or functional derivative thereof having endolysin activity.

10. A chimeric lysin protein comprising:

(i) the polypeptide of item 8 or the endolysin protein of item 9 and a heterologous protein, wherein the chimeric lysin protein has lysin activity; or

(ii) the polypeptide of item 8 or the endolysin protein of item 9, wherein the enzymatically active domain (EAD) of the polypeptide of item 8 or the endolysin protein of item 9 is substituted with an EAD of a heterologous lysin protein, wherein the chimeric lysin has lysin activity.

11. A composition, preferably a pharmaceutical composition or a disinfecting composition, comprising (i) the bacteriophage of item 1 or 2, (ii) the nucleic acid molecule of item 3, (iii) the vector of item 4, (iv) the host cell of item 5 or 6, (v) the polypeptide of item 8, (vi) the endolysin protein of item 9, or (vii) the chimeric lysin of item 10.

12. A solution, preferably a disinfecting solution, comprising (i) the bacteriophage of item 1 or 2, (ii) the nucleic acid molecule of item 3, (iii) the vector of item 4, (iv) the host cell of item 5 or 6, (v) the polypeptide of item 8, (vi) the endolysin protein of item 9, or (vii) the chimeric lysin of item 10.

13. A method for controlling *Listeria* contamination, preferably for sanitizing and/or disinfecting *Listeria* contamination, comprising applying the composition according to item 11 or the solution of item 12 to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method.

14. Use of (i) the bacteriophage of item 1 or 2, (ii) the nucleic acid molecule of item 3, (iii) the vector of item 4, (iv) the host cell of item 5 or 6, (v) the polypeptide of item 8, (vi) the endolysin protein of item 9, or (vii) the chimeric lysin of item 10 in a method for controlling *Listeria* contamination, preferably for sanitizing and/or disinfecting *Listeria* contamination, with the proviso that the method is not a therapeutic method.

15. The bacteriophage of item 1 or 2, the nucleic acid molecule of item 3, the vector of item 4, the host cell of item 5 or 6, the polypeptide of item 8, the endolysin protein of item 9, or the chimeric lysin of item 10 for use in the treatment and/or prevention of a *Listeria* infection.

16. A kit comprising (i) the bacteriophage of item 1 or 2, (ii) the nucleic acid molecule of item 3, (iii) the vector of item 4, (iv) the host cell of item 5 or 6, (v) the polypeptide of item 8, (vi) the endolysin protein of item 9, or (vii) the chimeric lysin of item 10.

17. An antibody or fragment thereof that binds specifically to the polypeptide of item 8, the endolysin protein of item 9, or the chimeric lysin protein of item 10.

18. A nucleic acid molecule comprising the DNA sequence of the genome of the bacteriophage of item 1 or 2.

19. A product comprising (i) the bacteriophage of item 1 or 2, (ii) the polypeptide of item 8, (iii) the endolysin protein of item 9, or (iv) the chimeric lysin of item 10.

20. The product of item 19, which is a food product, preferably a dairy product.

21. A bacteriophage having lytic activity against *Listeria* serovar 3 obtainable by (a) plating a sample containing bacteriophage and *Listeria* bacteria serovar 3 to obtain plaques, and (b) purifying the phage contained within the one or more plaques.

22. A bacteriophage, which has lytic activity against *Listeria* serovar 3.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows trypsin digestion of endolysins PlyP825, Ply511 and PlyP40. Aliquots were analyzed at 0 and 3 min. The marker is shown in kDa. Analysis was performed on a 4-12% SDS-Gel (NuPage Novex, Invitrogen).

FIG. **2** shows MBC (minimum bactericidal concentration) testing of endolysins PlyP825, Ply511 and PlyP40 in buffer pH 6 against *Listeria monocytogenes* EGDe sv 1/2a and *Listeria innocua* WSLC2011 sv 6a. Buffer: 20 mM sodium phosphate, 50 mM sodium chloride, 0.05% Tween pH 6.

FIG. **3** shows MBC testing of endolysins PlyP825, Ply511 and PlyP40 in milk 1.5% fat against *Listeria monocytogenes* EGDe sv 1/2a and *Listeria* innocua WSLC2011 sv 6a. Testing was performed with 20 μg/ml endolysin.

FIG. **4** shows MBC testing of endolysins PlyP825, Ply511 and PlyP40 in milk 3.5% fat against *Listeria monocytogenes* EGDe sv 1/2a. Testing was performed with 20 μg/ml endolysin.

FIG. **5** shows a comparison of the relative lytic activity of endolysins Ply511, PlyP40, and PlyP825 as a function of the pH.

FIG. **6** shows a comparison of the relative lytic activity of endolysins Ply511, PlyP40, and PlyP825 as a function of the salt concentration.

FIG. **7** shows a comparison of the relative lytic activity of endolysins Ply511, PlyP40, and PlyP825 as a function of the EDTA concentration.

FIG. **8** shows a comparison of the MIC of endolysins Ply511, PlyP40, and PlyP825 against *Listeria monocytogenes* ProCC S1095 sv 1/2a, *Listeria monocytogenes* ProCC S1135 sv 3a, *Listeria monocytogenes* ProCC S776 sv 4b, and *Listeria innocua* ProCC S1147 sv 6a.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Bacteriophage-encoded endolysins are highly active enzymes, which hydrolyze bacterial cell walls. These phage-encoded cell wall lytic enzymes are synthesized late during virus replication and mediate the release of progeny virions. Endolysins can be used to lyse *Listeria* cells in various applications including *Listeria* contamination and infection. Endolysins can also be used to lyse *Listeria* cells simply to recover nucleic acids or cellular protein for detection or differentiation.

The novel *Listeria*-specific bacteriophage ProCC P825 ("P825") provided by the present invention has been deposited internationally on Jul. 14, 2010 at the DSMZ—

Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH, Braunschweig, Germany, under international deposit accession No. DSM No. 23783 in accordance with the Budapest treaty concerning deposit of cell cultures. The address of DSMZ is Inhoffenstr. 7B, 38124 Braunschweig, Germany.

The name and address of the depositor of the novel bacteriophage P825 is as follows: Hyglos Invest GmbH, Am Neuland 1, 82347 Bernried, Germany. Evidence is provided by a separate document enclosed with this application that the depositor Hyglos Invest GmbH, Bernried, Germany, has authorized the applicant to refer to the deposited biological material in the present application, and has given his unreserved and irrevocable consent to the deposited material being made available to the public (in accordance with, for example, Rule 33 EPC). In addition, said separate document provides evidence that the depositor Hyglos Invest GmbH, Bernried, Germany, has given his consent that the applicant makes use of the so-called "expert solution" (in accordance with, for example, Rule 32 EPC).

The novel *Listeria*-specific bacteriophages provided by the present invention are capable of lysing *Listeria* serovars 1/2, 3, 4, 5 and 6. Thus, bacteriophages according to the present invention are broad host range bacteriophages. Importantly, these novel phages are capable of lysing *Listeria* serovar 3, which is one of the clinically relevant *Listeria* serovars. Therefore, bacteriophages of the present invention are in particular those which are capable of lysing *Listeria* serovar 3. This activity is unique to the novel bacteriophages provided by the present invention. *Listeria*-specific bacteriophages described in the art do not exhibit this specific property. The novel bacteriophages provided by the present invention are strictly lytic and therefore invariably lethal to a *Listeria* bacterial cell after infection. The lytic activity comes from the endolysin encoded by the novel phages capable of lysing *Listeria* serovars 1/2, 3, 4, 5 and 6. Therefore, the endolysin encoded by the novel bacteriophages of the present invention can be used for controlling *Listeria* contamination and infection. The endolysins encoded by the novel bacteriophages comprise an EAD (enzymatically active domain), which contributes for the lytic activity of the endolysin, and a CBD (cell wall binding domain), which targets the lysin to its substrate.

In various embodiments, a novel *Listeria*-specific bacteriophage provided by the present invention is a non-modified bacteriophage capable of lysing *Listeria* serovars 1/2, 3, 4, 5 and 6, in particular a non-modified bacteriophage capable of lysing *Listeria* serovar 3. As used herein, a non-modified bacteriophage is a wild-type bacteriophage.

The novel *Listeria* bacteriophage P825 is capable of lysing *Listeria* serovars 1/2, 3, 4, 5 and 6. Importantly, phage P825 is capable of lysing *Listeria* serovar 3, which is one of the clinically relevant *Listeria* serovars. This activity is unique to the novel bacteriophage P825. Bacteriophages described in the art do not exhibit this specific property. The novel *Listeria* bacteriophage P825 is strictly lytic and therefore invariably lethal to a *Listeria* bacterial cell after infection. The lytic activity comes from the endolysin PlyP825 encoded by the phage P825. Therefore, PlyP825 can be used for controlling *Listeria* contamination and infection. PlyP825 comprises an EAD (enzymatically active domain), which contributes for the lytic activity of the endolysin, and a CBD (cell wall binding domain), which targets the lysin to its substrate. The nucleotide and amino acid sequence of the PlyP825 EAD are shown in SEQ ID NOs: 3 and 4, respectively. The nucleic acid sequence encoding the PlyP825 EAD comprises nucleotides 1 to 426 of SEQ ID NO: 1. The amino acid sequence of the PlyP825 EAD comprises amino acid residues 1 (M1) to 142 (E142) of SEQ ID NO: 2. In the present invention, the EAD of SEQ ID NO: 4 may also be called "the lytic domain" of the PlyP825 endolysin of SEQ ID NO: 2.

The nucleotide and amino acid sequence of the PlyP825 CBD are shown in SEQ ID NOs: 5 and 6, respectively. The nucleic acid sequence encoding the PlyP825 CBD comprises nucleotides 487 to 945 of SEQ ID NO: 1. The amino acid sequence of the PlyP825 CBD comprises amino acid residues 163 (G163) to 315 (N315) of SEQ ID NO: 2. In the present invention, the CBD of SEQ ID NO: 4 may also be called "the cell wall binding domain" of the PlyP825 endolysin of SEQ ID NO: 2.

The nucleotide sequence of the genome of phage P825 is depicted in SEQ ID NO: 7, and contains 66,849 nucleotides, including the stop codon.

Lytic Activity of Phages of the Present Invention

The phages provided by the present invention exhibit lytic activity against *Listeria* bacteria. As demonstrated by the inventors, phage P825 completely inhibited growth of *Listeria monocytogenes* strains. Phage P825 not only inhibited growth but actually reduced *Listeria* titers. As confirmed by enrichment studies, applying phage P825 completely eradicated *Listeria* bacteria. The lysis spectrum of phage P825 has been shown to be consistent with the host specificity provided by the tail spike protein of phage P825 responsible for receptor binding on the *Listeria* cell surface.

The present invention provides bacteriophages capable of lysing *Listeria* serovars 1/2, 3, 4, 5, and 6. A preferred phage is phage P825. The present invention also provides phages that are capable of lysing *Listeria* serovars 1/2, 3, 4, 5, 6 and 7. A preferred phage is phage P825. In various embodiments, a phage according to the present invention is capable of specifically lysing *Listeria* serovar 3. A preferred phage is phage P825.

As described above, the phages provided by the present invention exhibit lytic activity against *Listeria* bacteria, i.e., they have the activity of lysing *Listeria* bacteria, in particular *Listeria* serovars 1/2, 3, 4, 5, 6 and 7, in particular *Listeria* serovar 3. In the context of the present invention, the terms "exhibiting lytic activity against *Listeria* bacteria", "having lytic activity against *Listeria* bacteria", "having the activity of lysing *Listeria* bacteria" and "being capable of lysing *Listeria* bacteria" may be used interchangeably. The present invention provides a bacteriophage capable of lysing *Listeria* bacteria, preferably *Listeria monocytogenes*, wherein the bacteriophage has a genome (i) comprising the DNA sequence of SEQ ID NO: 7; (ii) having at least 90% or 95% sequence identity with the DNA sequence of SEQ ID NO: 7; or (iii) having at least 90% or 95% sequence identity with the DNA sequence of the genome of bacteriophage ProCC P825 deposited under accession No. DSM 23783. In various embodiments, the phage provided by the present invention is capable of lysing any one of the *Listeria* species described herein. In various embodiments, the phage according to the present invention has a genome having at least 96%, 97%, 98%, or 99% sequence identity with the DNA sequence of the genome of bacteriophage ProCC P825 deposited under accession No. DSM 23783. In various embodiments, the phage according to the present invention has a genome having at least 96%, 97%, 98%, or 99% sequence identity with the DNA sequence of SEQ ID NO: 7. Preferably, the phage according to the present invention is bacteriophage ProCC P825 deposited under accession No. DSM 23783.

The present invention provides a nucleic acid molecule comprising the DNA sequence of the genome of a bacteriophage according to the present invention. In various embodiments, the nucleic acid molecule comprises the DNA sequence of SEQ ID NO: 7. In various embodiments, the nucleic acid molecule has at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the DNA sequence of SEQ ID NO: 7. In various embodiments, the nucleic acid molecule has at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the DNA sequence of the genome of bacteriophage ProCC P825 deposited under accession No. DSM 23783. The present invention provides any polypeptide encoded by the nucleic acid molecule of SEQ ID NO: 7 or variants thereof as described herein above.

Lytic Activity of Proteins of the Invention

A major problem of phage endolysins is the proteolytic instability. Until now, two *Listeria* endolysins are known that are not restricted to lyse distinct *Listeria* serovars like Ply500 and Ply118 (Loessner et al., 2002), but are able to lyse several *Listeria* serovars: Ply511 of *Listeria* phage A511 and PlyP40 of *Listeria* phage P40. However, a bacteriophage according to the present invention is capable of lysing all of serovars 1/2, 3, 4, 5, and 6. This property is unique to bacteriophages of the present invention. Thus, bacteriophages according to the present invention are broad host range bacteriophages. Importantly, bacteriophages of the present invention are capable of lysing *Listeria* serovar 3, which is one of the clinically relevant serovars. This activity is not shared by any known *Listeria*-specific bacteriophages.

In order to compare the proteolytic sensitivity of the three endolysins of phages A511, P40, and P825, they were Trypsin-digested in equimolar amounts. Aliquots were retained and analyzed after 0 and 3 min incubation at room temperature (FIG. **1**). As shown in FIG. **1**, PlyP825 shows less proteolytic degradation sites than Ply511 and PlyP40.

PlyP825 was analyzed for its activity against different *Listeria* strains with serovars 1/2, 3, 4, 5 and 6. Exponential *Listeria* cells were poured in LB-Top Agar in plates. Onto the solidified agar 2 μg of PlyP825 was spotted. After incubation over night at 30° C. all 22 strains tested were lysed by the endolysin PlyP825 (Table 2). Thus, PlyP825 is a broad range *Listeria* endolysin.

The minimum bactericidal concentrations (MBC) of endolysins PlyP825, Ply511 and PlyP40 in buffer and in milk were determined and compared. For determining the MBC in buffer pH 6 the endolysin enzymes were incubated with 10^5 cells/ml of strains *Listeria monocytogenes* EGDe sv 1/2a and *Listeria* innocua WSLC2011 sv 6a in buffer (20 mM Sodium-phosphate, 50 mM sodium chloride, 0.05% Tween 20 pH 6) at 30° C. After 1 h the samples were plated and cell numbers counted. FIG. **2** shows the results: PlyP825 reduces effectively pathogenic and non-pathogenic *Listeria* cells in buffer: 0.032 μg/ml endolysin were sufficient to reduce 4.5 (WSLC2011) or 3.1 (EGDe) orders of magnitude of *Listeria* cells. This is about 0.5 to 1.5 log more than Ply511 and 0.9-1.3 log more than PlyP40 were able to reduce with the same protein concentration.

For determining the MBC in milk the enzymes were incubated with 10^5 cells/ml of strains *Listeria monocytogenes* EGDe sv 1/2a and *Listeria* innocua WSLC2011 sv 6a in milk with 1.5% fat at 30° C. After 3 h the samples were plated and cell numbers counted. FIG. **3** shows the results: PlyP825 shows the highest *Listeria* cell reduction in milk. Independent from the test strain PlyP825 reduces 1.4-1.7 orders of magnitude more cells than the other two broad *Listeria* endolysins Ply511 and PlyP40 in milk with 1.5% fat.

Besides the enzymes were incubated with 10^5 cells/ml of strains *Listeria monocytogenes* EGDe sv 1/2a in milk with 3.5% fat at 30° C. After 3 h the samples were plated and cell numbers counted. FIG. **4** shows the results: Also in milk with 3.5% fat PlyP825 reduces the highest cell number.

PlyP825 Nucleic Acid and Amino Acid Sequences and Variants Thereof

The present invention provides a nucleic acid molecule comprising a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2. The present invention also provides a nucleic acid molecule, which comprises a polynucleotide that is at least 75% or at least 80% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. The present invention further provides a nucleic acid molecule, which comprises a polynucleotide that is at least 85% or at least 90% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. In various embodiments, the said nucleic acid molecule comprises a polynucleotide that is at least 91% or at least 92% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. Preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 93% or at least 94% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. More preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 95% or at least 96% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. Still more preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 97%, at least 98%, or even at least 99% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 75% or at least 80% identical to the amino acid sequence of SEQ ID NO: 2, and which has endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. The present invention also provides a nucleic acid molecule comprising a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 85% or at least 90% identical to the amino acid sequence of SEQ ID NO: 2, and which has endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. In various embodiments, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 91% or at least 92% identical to the amino acid sequence of SEQ ID NO: 2, and which has endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. Preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 93% or at least 94% identical to the amino acid sequence of SEQ ID NO: 2, and which has endolysin activity, preferably the activity of the

endolysin of SEQ ID NO: 2. More preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 95% or at least 96% identical to the amino acid sequence of SEQ ID NO: 2, and which has endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. Still more preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 97%, at least 98%, or even 99% identical to the amino acid sequence of SEQ ID NO: 2, and which has endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide, which encodes a fragment, analog or functional derivative of a polypeptide encoded by a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein said fragment, analog or functional derivative has endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. Preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a fragment, analog or functional derivative of a polypeptide encoded by the polynucleotide of SEQ ID NO: 1, wherein said fragment, analog or functional derivative has endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2.

The present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to any one of the polynucleotides described in the three preceding paragraphs. The present invention also provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of any one of the polynucleotides described in the three preceding paragraphs.

The present invention provides a nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of SEQ ID NO: 1. The present invention also provides a nucleic acid molecule, which comprises a polynucleotide that is at least 75% or at least 80% identical to the nucleotide sequence of SEQ ID NO: 1, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. The present invention further provides a nucleic acid molecule, which comprises a polynucleotide that is at least 85% or at least 90% identical to the nucleotide sequence of SEQ ID NO: 1, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. In various embodiments, the said nucleic acid molecule comprises a polynucleotide that is at least 91% or at least 92% identical to the nucleotide sequence of SEQ ID NO: 1, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. Preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 93% or at least 94% identical to the nucleotide sequence of SEQ ID NO: 1, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. More preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 95% or at least 96% identical to the nucleotide sequence of SEQ ID NO: 1, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2. Still more preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 97%, at least 98%, or even at least 99% identical to the nucleotide sequence of SEQ ID NO: 1, and that encodes a polypeptide having endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide that is a part of the nucleotide sequence of SEQ ID NO: 1, and that encodes a fragment, analog or functional derivative of the polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein said fragment, analog or functional derivative has endolysin activity, preferably the activity of the endolysin of SEQ ID NO: 2.

The present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to any one of the polynucleotides described in the two preceding paragraphs. Preferably, the present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to the polynucleotide of SEQ ID NO: 1. The present invention also provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of any one of the polynucleotides described in the two preceding paragraphs. Preferably the present invention provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of the polynucleotide of SEQ ID NO: 1.

PlyP825 EAD Nucleic Acid and Amino Acid Sequences and Variants Thereof

The present invention provides a nucleic acid molecule comprising a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 4. The present invention also provides a nucleic acid molecule, which comprises a polynucleotide that is at least 75% or at least 80% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 4, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. The present invention further provides a nucleic acid molecule, which comprises a polynucleotide that is at least 85% or at least 90% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 4, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. In various embodiments, the said nucleic acid molecule comprises a polynucleotide that is at least 91% or at least 92% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 4, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. Preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 93% or at least 94% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 4, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. More preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 95% or at least 96% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 4, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. Still more preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 97%, at least 98%, or even at least 99% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 4, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 75% or at least 80% identical to the amino acid sequence of SEQ ID NO: 4, and which has the lytic activity of the EAD of SEQ ID NO: 4. The present invention also provides a nucleic acid molecule comprising a polynucleotide, which encodes a polypeptide having an amino acid sequence that

is at least 85% or at least 90% identical to the amino acid sequence of SEQ ID NO: 4, and which has the lytic activity of the EAD of SEQ ID NO: 4. In various embodiments, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 91% or at least 92% identical to the amino acid sequence of SEQ ID NO: 4, and which has the lytic activity of the EAD of SEQ ID NO: 4. Preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 93% or at least 94% identical to the amino acid sequence of SEQ ID NO: 4, and which has the lytic activity of the EAD of SEQ ID NO: 4. More preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 95% or at least 96% identical to the amino acid sequence of SEQ ID NO: 4, and which has the lytic activity of the EAD of SEQ ID NO: 4. Still more preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 97%, at least 98%, or even 99% identical to the amino acid sequence of SEQ ID NO: 4, and which has the lytic activity of the EAD of SEQ ID NO: 4.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide, which encodes a fragment, analog or functional derivative of a polypeptide encoded by a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 4, wherein said fragment, analog or functional derivative has the lytic activity of the EAD of SEQ ID NO: 4. Preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a fragment, analog or functional derivative of a polypeptide encoded by the polynucleotide of SEQ ID NO: 3, wherein said fragment, analog or functional derivative has the lytic activity of the EAD of SEQ ID NO: 4.

The present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to any one of the polynucleotides described in the three preceding paragraphs. The present invention also provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of any one of the polynucleotides described in the three preceding paragraphs.

The present invention provides a nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of SEQ ID NO: 3. The present invention also provides a nucleic acid molecule, which comprises a polynucleotide that is at least 75% or at least 80% identical to the nucleotide sequence of SEQ ID NO: 3, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. The present invention further provides a nucleic acid molecule, which comprises a polynucleotide that is at least 85% or at least 90% identical to the nucleotide sequence of SEQ ID NO: 3, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. In various embodiments, the said nucleic acid molecule comprises a polynucleotide that is at least 91% or at least 92% identical to the nucleotide sequence of SEQ ID NO: 3, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. Preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 93% or at least 94% identical to the nucleotide sequence of SEQ ID NO: 3, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. More preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 95% or at least 96% identical to the nucleotide sequence of SEQ ID NO: 3, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4. Still more preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 97%, at least 98%, or even at least 99% identical to the nucleotide sequence of SEQ ID NO: 3, and that encodes a polypeptide having the lytic activity of the EAD of SEQ ID NO: 4.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide that is a part of the nucleotide sequence of SEQ ID NO: 3, and that encodes a fragment, analog or functional derivative of the polypeptide having the amino acid sequence of SEQ ID NO: 4, wherein said fragment, analog or functional derivative has the lytic activity of the EAD of SEQ ID NO: 4.

The present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to any one of the polynucleotides described in the two preceding paragraphs. Preferably, the present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to the polynucleotide of SEQ ID NO: 3. The present invention also provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of any one of the polynucleotides described in the two preceding paragraphs. Preferably the present invention provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of the polynucleotide of SEQ ID NO: 3.

PlyP825 CBD Nucleic Acid and Amino Acid Sequences and Variants Thereof

The present invention provides a nucleic acid molecule comprising a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6. The present invention also provides a nucleic acid molecule, which comprises a polynucleotide that is at least 75% or at least 80% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. The present invention further provides a nucleic acid molecule, which comprises a polynucleotide that is at least 85% or at least 90% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. In various embodiments, the said nucleic acid molecule comprises a polynucleotide that is at least 91% or at least 92% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. Preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 93% or at least 94% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. More preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 95% or at least 96% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. Still more preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 97%, at least 98%, or even at least 99% identical to a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 75% or at least 80% identical to the amino acid sequence of SEQ ID NO: 6, and which has the cell wall binding activity of the CBD of SEQ ID NO: 6. The present invention also provides a nucleic acid molecule comprising a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 85% or at least 90% identical to the amino acid sequence of SEQ ID NO: 6, and which has the cell wall binding activity of the CBD of SEQ ID NO: 6. In various embodiments, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 91% or at least 92% identical to the amino acid sequence of SEQ ID NO: 6, and which has the cell wall binding activity of the CBD of SEQ ID NO: 6. Preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 93% or at least 94% identical to the amino acid sequence of SEQ ID NO: 6, and which has the cell wall binding activity of the CBD of SEQ ID NO: 6. More preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 95% or at least 96% identical to the amino acid sequence of SEQ ID NO: 6, and which has the cell wall binding activity of the CBD of SEQ ID NO: 6. Still more preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a polypeptide having an amino acid sequence that is at least 97%, at least 98%, or even 99% identical to the amino acid sequence of SEQ ID NO: 6, and which has the cell wall binding activity of the CBD of SEQ ID NO: 6.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide, which encodes a fragment, analog or functional derivative of a polypeptide encoded by a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6, wherein said fragment, analog or functional derivative has the cell wall binding activity of the CBD of SEQ ID NO: 6. Preferably, the said nucleic acid molecule comprises a polynucleotide, which encodes a fragment, analog or functional derivative of a polypeptide encoded by the polynucleotide of SEQ ID NO: 5, wherein said fragment, analog or functional derivative has the cell wall binding activity of the CBD of SEQ ID NO: 6.

The present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to any one of the polynucleotides described in the three preceding paragraphs. The present invention also provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of any one of the polynucleotides described in the three preceding paragraphs.

The present invention provides a nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of SEQ ID NO: 5. The present invention also provides a nucleic acid molecule, which comprises a polynucleotide that is at least 75% or at least 80% identical to the nucleotide sequence of SEQ ID NO: 5, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. The present invention further provides a nucleic acid molecule, which comprises a polynucleotide that is at least 85% or at least 90% identical to the nucleotide sequence of SEQ ID NO: 5, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. In various embodiments, the said nucleic acid molecule comprises a polynucleotide that is at least 91% or at least 92% identical to the nucleotide sequence of SEQ ID NO: 5, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. Preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 93% or at least 94% identical to the nucleotide sequence of SEQ ID NO: 5, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. More preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 95% or at least 96% identical to the nucleotide sequence of SEQ ID NO: 5, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6. Still more preferably, the said nucleic acid molecule comprises a polynucleotide that is at least 97%, at least 98%, or even at least 99% identical to the nucleotide sequence of SEQ ID NO: 5, and that encodes a polypeptide having the cell wall binding activity of the CBD of SEQ ID NO: 6.

Furthermore, the present invention provides a nucleic acid molecule comprising a polynucleotide that is a part of the nucleotide sequence of SEQ ID NO: 5, and that encodes a fragment, analog or functional derivative of the polypeptide having the amino acid sequence of SEQ ID NO: 6, wherein said fragment, analog or functional derivative has the cell wall binding activity of the CBD of SEQ ID NO: 6.

The present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to any one of the polynucleotides described in the two preceding paragraphs. Preferably, the present invention provides a nucleic acid molecule comprising a polynucleotide, which hybridizes under stringent conditions to the polynucleotide of SEQ ID NO: 5. The present invention also provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of any one of the polynucleotides described in the two preceding paragraphs. Preferably the present invention provides a nucleic acid molecule comprising a polynucleotide that is the complement of the full-length of the polynucleotide of SEQ ID NO: 5.

For a variant polypeptide of the present invention having an amino acid sequence at least, for example, 95% "identical" to the reference amino acid sequence of a reference polypeptide defined by a certain SEQ ID NO, is intended that the amino acid sequence of the variant polypeptide is identical to the reference amino acid sequence, except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the reference polypeptide shown in the respective SEQ ID NO. In other words, to obtain a variant polypeptide having an amino acid sequence at least 95% identical to the reference amino acid sequence of a certain reference SEQ ID NO, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the N-terminal or C-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of a reference SEQ ID NO can be determined conventionally using appropriate computer programs (i.e., sequence alignment programs) known in the art.

As used herein, a nucleic acid molecule of the present invention is DNA or RNA.

Vectors and Host Cells

The present invention provides recombinant vectors containing nucleic acid molecules of the present invention. In various embodiments, provided is a single recombinant vector containing a single nucleic acid molecule of the present invention. In various other embodiments, provided is a single recombinant vector containing several nucleic acid molecules of the present invention. In still other embodiments, provided are several recombinant vectors each containing a single nucleic acid molecule of the present invention. In still further embodiments, provided are several recombinant vectors each containing several nucleic acid molecule of the present invention.

In various embodiments, the nucleic acid molecule or nucleic acid molecules contained in a single or several vectors according to the present invention are operatively linked to an expression control sequence allowing expression of the polynucleotide or polynucleotides in prokaryotic or eukaryotic host cells. Preferably, the expression control sequence is a promoter or a promoter sequence. Suitable promoters are known to the skilled artisan. In various embodiments, the vector is a plasmid. Other suitable vectors will be readily apparent to the skilled artisan. A recombinant vector according to the present invention may also be called expression vector or expression construct.

The expression constructs according to the present invention may further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the EAD and/or CBD of the transcripts expressed by the constructs according to the present invention will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

In various embodiments, the expression vectors according to the present invention will include at least one selectable marker. Suitable selection markers are known to the skilled artisan.

The present invention provides a method of making a recombinant vector comprising inserting a nucleic acid molecule of the present invention into a vector.

The present invention also provides a method of making a recombinant host cell comprising introducing a nucleic acid molecule or a recombinant vector according to the present invention into a host cell.

The present invention also provides a host cell genetically engineered with a nucleic acid molecule or a recombinant vector according to the present invention. In various embodiments, "genetically engineered" means that the host cell is transformed or transfected with a nucleic acid molecule or a recombinant vector according to the present invention. In various embodiments, the genetically engineered host cell according to the present invention contains a polypeptide expressed from a nucleic acid molecule or from a recombinant vector in accordance with the present invention. Representative examples of appropriate host cells include, but are not limited to, bacterial cells such as *E. coli* cells, fungal cells such as yeast cells, insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells, animal cells such as CHO, COS, and HEK293 cells, and plant cells. Appropriate culture mediums and conditions for host cells of the present invention are known in the art.

Proteins/Polypeptides

Recombinant proteins of the present invention can be isolated and purified from a host cell of the present invention containing or expressing the proteins/polypeptides by techniques known in the art including, but not limited to, lysis, chromatography, filtration, and centrifugation. In various embodiments, the isolated and/or purified protein according to the present invention is labeled. Preferably, the label is selected from the group consisting of an enzyme label, a radioisotope, a fluorescent label, and biotin.

A protein of the present invention having lytic activity, preferably the PlyP825 endolysin, can be isolated from the host cell prior to administration in methods of controlling *Listeria* contamination and infection according to the present invention, or the host cell containing the recombinant protein can be directly applied or administered without prior isolation of the protein having lytic activity. For example, a host bacterium, which produces the PlyP825 endolysin of the present invention can be applied in methods of controlling *Listeria* contamination and infection according to the present invention where the endolysin would be secreted, for example, into food or foodstuff, onto a surface or in the gut of a subject. The PlyP825 endolysin of the present invention can then attack *Listeria* cells present in such an environment.

The present invention also provides a method of making a polypeptide of the present invention encoded by a nucleic acid molecule of the present invention, wherein the method comprises (i) culturing a genetically engineered host cell of the present invention under conditions such that the polypeptide encoded by a nucleic acid molecule of the present invention is expressed, and (ii) recovering the polypeptide encoded by the nucleic acid molecule. The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For example, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage of the polypeptide. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides of the present invention for, inter alia, inducing secretion, improving stability and/or facilitating purification are familiar to the ones of ordinary skill and belong to routine techniques in the art. A preferred fusion protein comprises a heterologous region from an immunoglobulin that is useful to stabilize and purify proteins.

As one of skill in the art will appreciate, polypeptides of the present invention can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins may facilitate purification and may show an increased half-life in vivo.

For many proteins it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function or activity. Here, biological function/activity includes any function and activity of the proteins of the present invention described herein including, but not limited to, any lytic function/activity and cell wall binding function/activity described herein.

In the present invention, since the protein of SEQ ID NO: 2 is a member of the endolysin polypeptide family, deletions of C-terminal amino acids up to the Arg (R) residue at position 143 in SEQ ID NO: 2 retains the lytic activity of the endolysin protein, i.e. the lytic activity to *Listeria* bacterial cells. Accordingly, the present invention provides endolysin polypeptides having one or more residues deleted from the C-terminus of the amino acid sequence of the endolysin protein of SEQ ID NO: 2, up to the Arg residue at position

143 (R143) in the amino acid sequence of SEQ ID NO: 2, and polynucleotides encoding such polypeptides.

The present invention provides polypeptides encoded by the nucleic acid molecules of the present invention. The present invention also provides polypeptides obtainable by methods of making the polypeptides according to the present invention. Therefore, the present invention encompasses and provides each polypeptide that is encoded by any nucleic acid molecule of the present invention. Furthermore, the present invention encompasses and provides each polypeptide that is obtainable by any method of making the polypeptide according to the present invention.

Antibodies

The present invention also provides an antibody or fragment thereof that binds specifically to a polypeptide of the present invention. Preferably, the antibody specifically binds to the full-length polypeptide having the amino acid sequence of SEQ ID NO: 2, 4 or 6. In various embodiments, the antibody specifically binds to the lytic domain of the endolysin polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein the lytic domain comprises the amino acid sequence of residues 1 (M1) to 142 (E142) of SEQ ID NO: 2. In various embodiments, the antibody specifically binds to the cell wall binding domain of the endolysin polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein the cell wall binding domain comprises the amino acid sequence of residues 163 (G163) to 315 (N315) of SEQ ID NO: 2.

In various embodiments, the antibody of the present invention is selected from the group consisting of a monoclonal antibody, a polyclonal antibody, a chimeric antibody, a Fab fragment, a $F(ab')_2$ fragment, and a scFv fragment. In various embodiments, the antibody according to the present invention is labeled. Preferably, the label is selected from the group consisting of an enzyme label, a radioisotope, a fluorescent label, and biotin. The polypeptides of the present invention can be used to raise polyclonal and monoclonal antibodies provided by the present invention. The antibodies of the present invention may be prepared by any of a variety of methods available in the art and known to the skilled artisan.

The antibody fragments provided by the present invention, whether attached to other sequences or not, can also include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the antibody fragment is not significantly altered or impaired compared to the non-modified antibody or antibody fragment. These modifications can provide for some additional property, such as to remove/add amino acids capable of disulfide bonding. In any case, antibody fragments according to the present invention must possess a bioactive property, such as specific binding to its cognate antigen.

Functional or active regions of the antibodies or antibody fragments of the present invention may be identified by mutagenesis of a specific region of the protein, followed by expression and testing of the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can include site-specific mutagenesis of the nucleic acid encoding the antibody or antibody fragment.

Combinations of Proteins of the Invention with Known *Listeria*-Specific Phages

The present invention provides the combination of a protein of the present invention, preferably an endolysin protein, with one or more other *Listeria*-specific bacteriophages described in the art. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention. In various embodiments concerning the combination of a protein of the present invention with one or more *Listeria* bacteriophages known in the art the protein of the present invention is the lytic domain of an endolysin protein according to the present invention. Preferably, the lytic domain of an endolysin protein according to the present invention is an EAD according to the present invention. In various other embodiments concerning the combination of a protein of the present invention with one or more *Listeria* bacteriophages known in the art the protein of the present invention is the cell wall binding domain of an endolysin protein according to the present invention. Preferably, the cell wall binding domain of an endolysin protein according to the present invention is a CBD to the present invention.

Combinations of Proteins of the Invention with Known Endolysins

Also provided by the present invention is the combination of a protein of the present invention, preferably an endolysin protein, with one or more endolysins from other *Listeria*-specific bacteriophages described in the art. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention. In various embodiments concerning the combination of a protein of the present invention with one or more endolysins from *Listeria* bacteriophages known in the art the protein of the present invention is the lytic domain of an endolysin protein according to the present invention. Preferably, the lytic domain of an endolysin protein according to the present invention is an EAD according to the present invention. In various other embodiments concerning the combination of a protein of the present invention with one or more endolysins from *Listeria* bacteriophages known in the art the protein of the present invention is the cell wall binding domain of an endolysin protein according to the present invention. Preferably, the cell wall binding domain of an endolysin protein according to the present invention is a CBD to the present invention.

Combinations of Proteins of the Invention with Known Lytic Domains

The present invention provides the combination of a protein of the present invention, preferably an endolysin protein, with one or more lytic domains of endolysins from other *Listeria*-specific bacteriophages described in the art. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention. Furthermore, based on sequence homology the skilled person is also able to determine the lytic domain of the endolysins encoded by known phages. In various embodiments concerning the combination of a protein of the present invention with one or more lytic domains of endolysins from *Listeria* bacteriophages known in the art the protein of the present invention is the lytic domain of an endolysin protein according to the present invention. Preferably, the lytic domain of an endolysin protein according to the present invention is an EAD according to the present invention. In various other embodiments concerning the combination of a protein of the present invention with one or more lytic domains of endolysins from *Listeria* bacteriophages known in the art the protein of the present invention is the cell wall binding domain of an endolysin protein according to the present invention. Preferably, the cell wall binding domain of an endolysin protein according to the present invention is a CBD to the present invention.

Also provided by the present invention is the combination of a protein of the present invention, preferably an endolysin protein, with one or more lytic domains of autolysins

described in the art. Autolysins are bacteriolytic enzymes that digest the cell-wall peptidoglycan of the bacteria that produce them. Autolysins are involved in cell wall reconstruction during bacterial cell division. Thus, the present invention provides a protein of the present invention, preferably an endolysin protein, in combination with one or more lytic domains of autolysins. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention.

Also provided by the present invention is the combination of a protein of the present invention, preferably an endolysin protein, with one or more lytic domains of bacteriocins described in the art. Bacteriocins are molecules also produced and secreted by microorganisms. They are antibacterial substances of a proteinaceous nature that are produced by different bacterial species. A subclass of bacteriocins consists of enzymes (proteinaceous toxins) which are produced by bacteria to inhibit the growth of similar or closely related concurrence bacterial strain(s) in their habitat. Many bacteria produce antimicrobial bacteriocin peptides. Thus, the present invention provides a protein of the present invention, preferably an endolysin protein, in combination with one or more lytic domains of bacteriocins. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention. Based on sequence homology the skilled person is able to determine the lytic domain of bacteriocins known in the art.

Also provided by the present invention is the combination of a protein of the present invention, preferably an endolysin protein, with one or more antimicrobial peptides. Antimicrobial peptides are ubiquitous, gene-encoded natural antibiotics that have gained recent attention in the search for new antimicrobials to combat infectious disease. Antimicrobial peptides generally have a length between 12 and 50 amino acids. The amphipathicity of the antimicrobial peptides allows to partition into the membrane lipid bilayer. The ability to associate with membranes is a definitive feature of antimicrobial peptides. Thus, the present invention provides a protein of the present invention, preferably an endolysin protein, in combination with one or more antimicrobial peptides. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention.

Combinations of Proteins of the Invention with Known Cell Wall Binding Domains

The present invention provides the combination of a protein of the present invention, preferably an endolysin protein, with one or more cell wall binding domains of endolysins from other *Listeria* bacteriophages described in the art. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention. As for the lytic domain encoded by the endolysins from known phages, based on sequence homology the skilled person is also able to determine the cell wall binding domain of the endolysins encoded by known phages. In various embodiments concerning the combination of a protein of the present invention with one or more cell wall binding domains of endolysins from *Listeria* bacteriophages known in the art the protein of the present invention is the lytic domain of an endolysin protein according to the present invention. Preferably, the lytic domain of an endolysin protein according to the present invention is an EAD according to the present invention. In various other embodiments concerning the combination of a protein of the present invention with one or more cell wall binding domains of endolysins from *Listeria* bacteriophages known in the art the protein of the present invention is the cell wall binding domain of an endolysin protein according to the present invention. Preferably, the cell wall binding domain of an endolysin protein according to the present invention is a CBD to the present invention.

Also provided by the present invention is the combination of a protein of the present invention, preferably an endolysin protein, with one or more cell wall binding domains of autolysins known in the art. Thus, the present invention provides a protein of the present invention, preferably an endolysin protein, in combination with one or more cell wall binding domains of autolysins. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention. Based on sequence homology the skilled person is able to determine the cell wall binding domain of autolysins known in the art.

Also provided by the present invention is the combination of a protein of the present invention, preferably an endolysin protein, with one or more cell wall binding domains of bacteriocins described in the art. Thus, the present invention provides a protein of the present invention, preferably an endolysin protein, in combination with one or more cell wall binding domains of bacteriocins. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention. Based on sequence homology the skilled

Chimeric Proteins

The present invention further provides a chimeric protein comprising a protein according to the present invention, preferably an endolysin protein of the present invention, and one or more heterologous proteins. Preferably, the chimeric protein of the present invention has endolysin activity. More preferably, the chimeric protein has the endolysin activity of the polypeptide of SEQ ID NO: 2. In various embodiments, the heterologous protein is a heterologous endolysin protein. In various embodiments, the chimeric protein according to the present invention comprises the lytic domain of an endolysin of the present invention and one or more heterologous proteins, wherein the lytic domain has the lytic activity of the EAD of SEQ ID NO: 4. Preferably, the lytic domain is the lytic domain of the endolysin of SEQ ID NO: 2 or the EAD of SEQ ID NO: 4. In various other embodiments, the chimeric protein according to the present invention comprises the cell wall binding domain of an endolysin of the present invention and one or more heterologous proteins, wherein the cell wall binding domain has the cell wall binding activity of the EAD of SEQ ID NO: 6. Preferably, the cell wall binding domain is the cell wall binding domain of the endolysin of SEQ ID NO: 2 or the CBD of SEQ ID NO: 6.

The present invention also provides a chimeric protein comprising an endolysin protein according to the present invention, wherein a catalytic domain of the endolysin protein is substituted with a catalytic domain of a heterologous endolysin protein, wherein the chimeric protein has endolysin activity. Preferably, such a chimeric protein has the endolysin activity of the polypeptide of SEQ ID NO: 2. In various embodiments, the catalytic domain of the endolysin protein of the present invention is the lytic domain, i.e., the EAD, and the catalytic domain of the heterologous endolysin protein is also its lytic domain. Accordingly, in various embodiments, the present invention provides a chimeric protein comprising an endolysin protein according to the present invention, wherein the lytic domain (i.e., the EAD) is substituted with the lytic domain of a heterologous endolysin protein, wherein the chimeric protein has endolysin activity. Preferably, the chimeric protein has the endolysin activity of the polypeptide of SEQ ID NO: 2. In various

embodiments, the present invention provides a chimeric protein comprising an endolysin protein according to the present invention, wherein the cell wall binding domain (i.e., the CBD) is substituted with the cell wall binding domain of a heterologous endolysin protein, wherein the chimeric protein has endolysin activity. Preferably, such a chimeric protein has the endolysin activity of the polypeptide of SEQ ID NO: 2.

The present invention further provides a chimeric protein comprising an endolysin protein of the present invention and one or more lytic domains (i.e., EADs) and/or one or more cell wall binding domains (i.e., CBDs) of other known endolysins from *Listeria* bacteriophages known in the art.

The present invention also provides a chimeric protein comprising a lytic domain of the present invention and one or more lytic domains (i.e., EADs) and/or one or more cell wall binding domains (i.e., CBDs) of other known endolysins from *Listeria* bacteriophages known in the art.

The present invention also provides a chimeric protein comprising a cell wall binding domain of the present invention and one or more lytic domains (i.e., EADs) and/or one or more cell wall binding domains (i.e., CBDs) of other known endolysins from *Listeria* bacteriophages known in the art.

In various embodiments, the chimeric proteins according to the present invention comprise more than one endolysin protein of the present invention. That is, the chimeric proteins according to the present invention may comprise tandem repeats of an endolysin protein of the present invention. Furthermore, in various embodiments the chimeric proteins according to the present invention comprise more than one lytic domain of the present invention. That is, the chimeric proteins according to the present invention may comprise one or more tandem repeats of a lytic domain of the present invention. Still further, in various embodiments the chimeric proteins according to the present invention comprise more than one cell wall binding domain of the present invention. That is, the chimeric proteins according to the present invention may comprise one or more tandem repeats of a cell wall binding domain of the present invention.

The present invention also provides chimeric proteins comprising the combination of a protein of the present invention with one or more endolysins from known *Listeria*-specific bacteriophages as described above.

The present invention also provides chimeric proteins comprising the combination of a protein of the present invention with one or more lytic domains from known endolysins as described above.

The present invention also provides chimeric proteins comprising the combination of a protein of the present invention with one or more lytic domains from known autolysins as described above.

The present invention also provides chimeric proteins comprising the combination of a protein of the present invention with one or more lytic domains from known bacteriocins as described above.

The present invention also provides chimeric proteins comprising the combination of a protein of the present invention with one or more antimicrobial peptides as described above.

The present invention also provides chimeric proteins comprising the combination of a protein of the present invention with one or more cell wall binding domains from known endolysins as described above.

The present invention also provides chimeric proteins comprising the combination of a protein of the present invention with one or more cell wall binding domains from known autolysins as described above.

The present invention also provides chimeric proteins comprising the combination of a protein of the present invention with one or more cell wall binding domains from known bacteriocins as described above.

Combinations of Phages of the Invention with Known Phages

The present invention provides the combination of a phage of the present invention with one or more bacteriophages, preferably known *Listeria*-specific phages, described in the art. Such combinations can be used for controlling *Listeria* contamination and/or infection according to the present invention. A preferred phage used in phage combinations according to the present invention is phage P825.

Compositions and Solutions

The present invention provides compositions comprising phage combinations and/or protein combinations of the invention as described herein above. Specifically, such a combination is the combination of a protein of the present invention with one or more known *Listeria* bacteriophages. Furthermore, such a combination is particularly the combination of a protein of the present invention with one or more endolysins from known *Listeria* bacteriophages. Such a combination is also particularly the combination of a protein of the present invention with one or more lytic or cell wall binding domains of endolysins from known *Listeria* bacteriophages. Still further, such a combination is the combination of a phage of the present invention, preferably phage P825, with one or more known *Listeria* bacteriophages.

The present invention also provides compositions comprising chimeric proteins according to the present invention. In general, the present invention provides a composition comprising a protein or polypeptide according to the present invention. The present invention also provides a composition comprising a nucleic acid molecule or a vector according to the present invention. The present invention further provides a composition comprising a host cell according to the present invention. The present invention further provides a composition comprising a protein or polypeptide according to the present invention. The present invention further provides a composition comprising a chimeric lysin according to the present invention. Still further, the present invention provides a composition comprising a phage of the present invention, preferably phage P825.

In various embodiments, a composition of the present invention further comprises listeriolysin, a surface disinfectant, an antibiotic, a surfactant, a lytic enzyme, or a bacteriophage specific for bacterial contaminants other than *Listeria* bacteria.

In various embodiments, a composition according to the present invention is a pharmaceutical composition.

In various embodiments, a composition according to the present invention is a disinfecting composition.

In various embodiments, a composition according to the present invention is a diagnostic composition. A phage of the present invention, preferably phage P825, is suitable for detecting the presence of *Listeria* bacteria according to the present invention. Therefore, a diagnostic composition according to the present invention preferably comprises a phage of the present invention, more preferably phage P825.

In various embodiments, a composition of the present invention is an antibiotic for use in therapeutic and non-therapeutic applications according to the present invention.

The present invention provides solutions, preferably disinfecting solutions, comprising phage combinations and

protein combinations of the invention as described herein above. Specifically, such a combination is the combination of a protein of the present invention with one or more known *Listeria* bacteriophages. Furthermore, such a combination is particularly the combination of a protein of the present invention with one or more endolysins from known *Listeria* bacteriophages. Such a combination is also particularly the combination of a protein of the present invention with one or more lytic or cell wall binding domains of endolysins from known *Listeria* bacteriophages. Still further, such a combination is the combination of a phage of the present invention, preferably phage P825, with one or more known *Listeria* bacteriophages.

The present invention also provides solutions, preferably disinfecting solutions, comprising chimeric proteins according to the present invention. In general, the present invention provides a solution, preferably a disinfecting solution, comprising a phage or protein or polypeptide according to the present invention. The present invention also provides a solution, preferably a disinfecting solution, comprising a nucleic acid molecule or a vector according to the present invention. The present invention further provides a solution, preferably a disinfecting solution, comprising a host cell according to the present invention.

Products

The present invention provides products comprising chimeric proteins according to the present invention. In general, the present invention provides a product comprising a protein or polypeptide according to the present invention, including any fragments, analogs or functional derivatives thereof having endolysin activity. The present invention further provides a product comprising a chimeric lysin according to the present invention. Still further, the present invention provides a product comprising a phage of the present invention, preferably phage P825.

The present invention also provides products comprising phage combinations and/or protein combinations of the invention as described herein above. Specifically, such a combination is the combination of a protein of the present invention with one or more known *Listeria* bacteriophages. Furthermore, such a combination is particularly the combination of a protein of the present invention with one or more endolysins from known *Listeria* bacteriophages. Such a combination is also particularly the combination of a protein of the present invention with one or more lytic or cell wall binding domains of endolysins from known *Listeria* bacteriophages. Still further, such a combination is the combination of a phage of the present invention, preferably phage P825, with one or more known *Listeria* bacteriophages.

In various embodiments, a product of the present invention further comprises listeriolysin, a surface disinfectant, an antibiotic, a surfactant, a lytic enzyme, or a bacteriophage specific for bacterial contaminants other than *Listeria* bacteria.

In various embodiments, a product according to the present invention is a food product. Preferably, the food product is any of a dairy product, a fruit product and a vegetable product.

Methods for Controlling *Listeria* Contamination

The present invention provides a method for controlling *Listeria* contamination, preferably for sanitizing and/or disinfecting *Listeria* contamination, comprising applying a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention to the present invention to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method.

The present invention provides a method for controlling *Listeria* contamination, preferably for sanitizing and/or disinfecting *Listeria* contamination, comprising applying a phage or a composition or solution according to the present invention to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method.

The present invention provides a composition or solution according to the present invention for use in therapy and/or prophylaxis.

The present invention provides a phage according to the present invention for use in diagnosis. Preferably, the bacteriophage for use in diagnosis is phage P825.

In various embodiments, controlling *Listeria* contamination according to the present invention is sanitizing and/or disinfecting *Listeria* contamination.

In various embodiments, controlling *Listeria* contamination according to the present invention is non-therapeutically treating *Listeria* contamination. Preferably, treating *Listeria* contamination is eradicating or removing undesired colonization of *Listeria* bacteria.

In the present invention, "*Listeria* contamination" means "undesired *Listeria* contamination". In the present invention, undesired *Listeria* contamination includes, but is not limited to, contamination of pathogenic *Listeria* bacteria. Here, pathogenic means exhibiting pathogenicity to human beings and/or animals. *Listeria monocytogenes* is pathogenic to both human and animals. Therefore, in the present invention controlling *Listeria* contamination preferably is controlling *Listeria monocytogenes* contamination.

In various embodiments, controlling *Listeria* contamination is cleaning from *Listeria* contamination.

In various embodiments, controlling *Listeria* contamination according to the present invention is *Listeria* decontamination. As used herein, *Listeria* decontamination means that after applying a phage or a composition or solution according to the present invention to the site of *Listeria* contamination the number of *Listeria* bacteria is reduced compared to the number of *Listeria* bacteria prior to applying a phage or a composition or solution according to the present invention to the site of *Listeria* contamination. The same holds for applying a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention in *Listeria* decontamination.

The present invention provides a combined treatment for controlling *Listeria* contamination, which comprises applying a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention and a further/additional anti-*Listeria* agent to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method. The present invention also provides a combined treatment for controlling *Listeria* contamination, which comprises applying a phage or composition or solution according to the present invention and a further/additional anti-*Listeria* agent to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method. Here, the further/additional anti-*Listeria* agent preferably is a disinfectant, an antimicrobial agent effective against *Listeria* bacteria, an enzyme, or a surfactant. The group of such antimicrobial agents effective against *Listeria* bacteria includes, but is not limited to, vancomycin, danofloxacin, and neomycin. Furthermore, in case of an enzyme as further/

additional anti-*Listeria* agent to be used in the present invention, the group of suitable enzymes includes enzymes aiding in breaking up biofilms. Such enzymes are known in the art and include, but are not limited to, polysaccharide depolymerases and proteases. The surfactant is particularly useful to solubilize and remove dirt so that the *Listeria* bacteria are accessible to the lytic proteins of the present invention.

The further/additional anti-*Listeria* agent may be applied to the site of *Listeria* contamination before or after applying a phage or a composition or solution according to the present invention to the site of *Listeria* contamination. This applies in analogy to the use of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention in a combined treatment comprising a further/additional anti-*Listeria* agent as used herein.

The present invention further provides a combined treatment for controlling *Listeria* contamination, which comprises a thermal treatment of the site of *Listeria* contamination, and subsequently applying a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method. The present invention further provides a combined treatment for controlling *Listeria* contamination, which comprises a thermal treatment of the site of *Listeria* contamination, and subsequently applying a phage or a composition or solution according to the present invention to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method. Specifically, thermal treatment of the site of *Listeria* contamination is heat treatment of the site of *Listeria* contamination, more preferably heat treatment at a temperature of at least 70° C., or 71° C. Still more preferably, thermal treatment is heat treatment at a temperature of at least 72° C., or 73° C. Even more preferably, thermal treatment is heat treatment at a temperature of at least 74° C., or 75° C.

The present invention provides a combined treatment for controlling *Listeria* contamination, which comprises applying a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention and an irradiation treatment of the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method. The present invention also provides a combined treatment for controlling *Listeria* contamination, which comprises applying a phage or a composition or solution according to the present invention and an irradiation treatment of the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method. As used herein, irradiation treatment means subjecting the site of *Listeria* contamination to ionizing radiation, also called ionizing energy. The radiation used to treat the site of *Listeria* contamination may be applied before or after a phage or a composition or solution according to the present invention is applied to the site of *Listeria* contamination. This applies in analogy to the use of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention in a combined treatment comprising irradiation treatment as used herein.

The present invention provides a combined treatment for controlling *Listeria* contamination, which comprises applying a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention and high intensity light emission treatment to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method. The present invention also provides a combined treatment for controlling *Listeria* contamination, which comprises applying a phage or a composition or solution according to the present invention and high intensity light emission treatment to the site of *Listeria* contamination, with the proviso that the method is not a therapeutic method. Specifically, high intensity light emission treatment may be performed by a pulsed power source, as described in MacGregor et al. 1998 ("Light inactivation of food-related pathogenic bacteria using a pulsed power source", *Letters in Applied Microbiology* 27(2):67-70). The high intensity light emission treatment may be applied to the site of *Listeria* contamination before or after a phage or a composition or solution according to the present invention is applied to the site of *Listeria* contamination. This applies in analogy to the use of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention in a combined treatment comprising high intensity light emission treatment as used herein.

A nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention can be applied on or into food or food products. Similarly, phages, compositions and solutions of the present invention can be applied on or into food or food products. Therefore, in various embodiments controlling *Listeria* contamination, preferably sanitizing and/or disinfecting *Listeria* contamination, is controlling *Listeria* contamination of food or a food product.

In various embodiments, controlling *Listeria* contamination, preferably sanitizing and/or disinfecting *Listeria* contamination, is controlling *Listeria* contamination of a solid surface. In various embodiments, such a solid surface is the surface of a food package, a food storage container or food processing equipment. The surface of food processing equipment includes the various physical sites within the food processing facilities/equipment.

The present invention provides food or a food product comprising a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention. The present invention also provides food or a food product comprising a phage or a composition or solution according to the present invention.

The present invention further provides a food package or food storage container comprising a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention. The present invention still further provides a food package or food storage container comprising a phage or a composition or solution according to the present invention. In various embodiments, the nucleic acid molecule, vector, host cell, protein or polypeptide, or chimeric lysin of the present invention is introduced into the food package or food storage container prior to sealing the food package or food

storage container. In various embodiments, a phage or composition or solution according to the present invention is introduced into the food package or food storage container prior to sealing the food package or food storage container. In various embodiments, the food, food product, food package or food storage container further comprises a further/additional anti-microbial agent. Here, the further/additional antimicrobial agent preferably is an antimicrobial agent effective against *Listeria* bacteria or other pathogenic bacteria. In various embodiments, the food, food product, food package or food storage container of the present invention has undergone thermal treatment prior to introducing the nucleic acid molecule, vector, host cell, protein or polypeptide, or chimeric lysin of the present invention to the food, food product, food package or food storage container. In various embodiments, the food, food product, food package or food storage container of the present invention has undergone thermal treatment prior to introducing a phage or the composition or solution of the present invention to the food, food product, food package or food storage container. Specifically, thermal treatment of the food, food product, food package or food storage container of the present invention is heat treatment of the food, food product, food package or food storage container of the present invention, more preferably heat treatment at a temperature of at least 70° C., or 71° C. Still more preferably, thermal treatment is heat treatment at a temperature of at least 72° C., or 73° C. Even more preferably, thermal treatment is heat treatment at a temperature of at least 74° C., or 75° C.

In the present invention, applying a phage or a composition or solution according to the present invention to the site of *Listeria* contamination includes incubating the site of *Listeria* contamination with a phage or a composition or solution according to the present invention. This applies in analogy to the use of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention as used herein. In the present invention, applying a phage or a composition or solution according to the present invention to the site of *Listeria* contamination also includes administering a phage or a composition or solution according to the present invention to the site of *Listeria* contamination. This applies in analogy to the use of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention as used herein.

The nucleic acid molecule, vector, host cell, protein or polypeptide, chimeric lysin, phage, composition or solution according to the present invention may be applied to the food, food product, food package or food storage container of the present invention by a number of means, including, but not limited to, admixing the nucleic acid molecule, vector, host cell, protein or polypeptide, chimeric lysin, phage, composition or solution into the food or food product, or spraying the nucleic acid molecule, vector, host cell, protein or polypeptide, chimeric lysin, phage, composition or solution according to the present invention into the food package or food storage container. Likewise, in the present invention the nucleic acid molecule, vector, host cell, protein or polypeptide, endolysin protein, chimeric lysin, phage, composition or solution according to the present invention may be applied to food processing facilities/equipment by a number of means including, but not limited to, spraying the nucleic acid molecule, vector, host cell, protein or polypeptide, endolysin protein, chimeric lysin, phage, composition or solution onto the food processing facilities/equipment and/or directly applying the nucleic acid molecule, vector, host cell, protein or polypeptide, chimeric lysin, phage, composition or solution to the food processing facilities/equipment. Said applications significantly reduce the numbers of *Listeria* bacteria.

The concentration of a protein according to the present invention, preferably an endolysin protein, for administration on or into food, food products, foodstuff and/or into various physical sites within food processing plants can be determined by one of skill in the art. That is, a suitable concentration is, for example, a concentration that provides for effectively controlling *Listeria* contamination according to the present invention. In various embodiments, the concentration is contemplated to be in the range of about 0.1-100 μg/ml, including the range of about 1-10 μg/ml and 0.5-5 μg/ml. In various embodiments, the concentration is contemplated to be in the range of about 1-5 μg/ml, 5-10 μg/ml, or 10-20 μg/ml. In various other embodiments, the concentration is contemplated to be in the range of about 20-40 μg/ml, 40-60 μg/ml, 60-80 μg/ml, or 80-100 μg/ml. The endolysin provided by the present invention can be applied in a liquid or a powdered form to food, food products, foodstuff, and/or food processing equipment. The nucleic acid molecule, vector, host cell, protein or polypeptide, chimeric lysin, phage, composition or solution of the present invention is administered until a successful reduction of the *Listeria* contamination is achieved or until the amount of *Listeria* bacteria is substantially reduced.

The present invention also provides the use of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, or a protein or polypeptide of the present invention in a non-therapeutic method for controlling *Listeria* contamination according to the present invention as described herein above.

Methods for Controlling *Listeria* Infection

The present invention provides a method for treating and/or preventing *Listeria* infection of a subject comprising administering a phage or a composition or solution of the present invention to the subject.

The present invention also provides a method for treating and/or preventing *Listeria* infection of a subject comprising administering a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention to the subject.

The methods for treating and/or preventing *Listeria* infection according to the present invention include treating and/or preventing a disease or condition caused by a *Listeria* infection. In various embodiments, the disease or condition caused by a *Listeria* infection is listeriosis. Listeriosis is an infection resulting form the ingestion of food or foodstuff contaminated by *Listeria* bacteria. In various embodiments listeriosis is caused by a *Listeria* infection resulting form the ingestion of food or foodstuff contaminated by *Listeria*. Preferably, listeriosis according to the present invention is caused by a *L. monocytogenes* infection resulting form the ingestion of food or foodstuff contaminated by *L. monocytogenes*. In various other embodiments the disease or condition caused by a *Listeria* infection is brain abscess, hepatitis, peritonitis, arthritis, gastroenteritis, encephalitis, sepsis, local wound infection, and inflammation of conjunctiva and cornea. Preferably, the disease or condition caused by a *Listeria* infection is listeriosis.

In various embodiments of the therapeutic methods of treatment according to the present invention the subject is a subject suffering from a *Listeria* infection or a subject supposed to suffer from a *Listeria* infection. In various embodiments of the therapeutic methods of treatment according to the present invention the subject is a subject at risk for a *Listeria* infection.

In various embodiments of the therapeutic methods of treatment according to the present invention the *Listeria* infection is a *Listeria monocytogenes* infection.

In various embodiments of the therapeutic methods of treating and/or preventing a disease or condition caused by a *Listeria* infection according to the present invention the disease or condition caused by a *Listeria* infection is a disease or condition caused by a *Listeria monocytogenes* infection.

In the present invention, the subject is a mammal including animals and human beings. In various embodiments, the subject preferably is a human being, more preferably a patient in need of a method for treating and/or preventing *Listeria* infection according to the present invention.

In various embodiments, the subject is a pregnant woman. In various other embodiments, the subject is a newborn baby. In various other embodiments, the subject is an elderly person, preferably a person of at least 60 years of age, more preferably a person of at least 65 years of age, still more preferably a person of at least 70 years of age. Even more preferably, the elderly person is a person of at least 75 years of age. In still more preferred embodiments, the elderly person is a person of at least 80 years of age.

The present invention provides a kit comprising a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention. The present invention also provides a kit comprising a phage or a composition or solution of the present invention. In various embodiments, the kit according to the present invention is a kit for use in a therapeutic or non-therapeutic method according to the present invention, or a kit for carrying out a therapeutic or non-therapeutic method according to the present invention. In various other embodiments, the kit according to the present invention is a kit for controlling *Listeria* contamination according to the present invention. In various embodiments, the kit is a diagnostic kit.

In various embodiments, the pharmaceutical composition according to the present invention comprises optionally a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" includes, but is not limited to, a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the medicament/pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. Pharmaceutical carriers are known to those skilled in the art. Suitable carriers and their formulations are described in Remington: The Science and Practice of Pharmacy (19^{th} ed.) ed. A. R. Gennaro, Mack Publishing Company, Easton, Pa. 1995. Typically, an appropriate amount of a pharmaceutically acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

In the therapeutic methods of treatment according to the present invention, the dosage or dosages to be administered to a subject will vary with the age, condition, sex and extent of the *Listeria* infection and/or disease or condition caused by a *Listeria* infection in the subject, route of administration, or whether other drugs are included in the regimen. The dosage or dosages to be administered to a subject can be determined by one of skill in the art. Furthermore, the dosage to be administered to a subject can be adjusted by the individual physician in the event of any counter indications.

Pharmaceutical compositions of the present invention may be administered by any suitable route of administration including, but not limited to, oral administration, rectal administration, parenteral administration, intravaginal administration, intraperitoneal administration, topical administration (as by powders, ointments, drops or transdermal patch), buccal administration, administration by inhalant or by nasal administration. As used herein, nasal administration, including topical intranasal administration, means delivery of a phage or a composition or solution of the present invention into the nose and nasal passages through one or both of the nares, and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of a phage or the composition or solution. This applies in analogy to the use of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention as used herein. Administration of a phage or the composition or solution by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. This applies in analogy to the use of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention as used herein.

The term "parenteral" as used herein refers to modes of administration, which include, but are not limited to, intravenous, intramuscular, intraperitoneal, subcutaneous and intra-articular injection and infusion.

In various embodiments, the dosage of administration for the phage P825 is contemplated to be in the range of about 10^3 to about 10^{13} pfu/per kg bodyweight/per day, preferably in the range of about 10^{12} pfu/per kg bodyweight/per day.

In various embodiments, the dosage of administration for the PlyP825 endolysin is contemplated to be in the range of about 2-2000 ng/per g bodyweight/per day, preferably in the range of about 20-200 ng/per g bodyweight/per day.

Pharmaceutical compositions according to the present invention may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the active ingredient of choice.

Formulations for topical administration of a phage or a composition or solution according to the present invention may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Also, formulations for topical administration of a nucleic acid molecule of the present invention, a vector of the present invention, a host cell of the present invention, a protein or polypeptide of the present invention, or a chimeric lysin of the present invention may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders.

The addition of conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable in formulations for topical administration of compositions according to the present invention.

In the present invention, food, foodstuff, and food products include, but are not limited to, dairy products, meat products, fish products, unpasteurized food products, fruits, vegetables and salads. As used herein, the term "dairy product" is intended to include any food product made using milk or milk products, including, but not limited to, milk, yoghurt, ice cream, cheese, butter, and cream. In various embodiments, the milk is raw milk or milk that has been pasteurized. As used herein, the term "meat product" is intended to include any food product, which contains animal tissue, including, but not limited to, beef, pork, and poultry. The term "ready to eat meat product" is intended to include any meat product, which does not require cooking prior to consumption, including, but not limited to, pates, hot dogs, bologna, salami, and cold cuts. As used herein, the term "fish product" is intended to include any food product, which contains tissue from an aquatic animal, including, but not limited to, lobster, crab, fresh water and saltwater fish and other seafoods. As used herein, the term "unpasteurized food product" is intended to include any food product, which is prepared using unpasteurized primary ingredients and which does not undergo a final (listeriocidal) heat treatment. As used herein, the term "salad" is intended to include any food product, which contains mixtures of vegetables or fruits, and particularly such mixtures as are presented for consumers to choose from in a display commonly referred to as a "salad bar".

Method for Detecting *Listeria* Bacteria

The present invention provides a method for detecting the presence of *Listeria* bacteria according to the present invention, preferably *Listeria monocytogenes*, comprising (i) providing a sample suspected to contain *Listeria* bacteria, preferably *L. monocytogenes*; (ii) incubating the sample of (i) with a phage or a composition according to the present invention, preferably a diagnostic composition; and (iii) detecting in the sample of (ii) the presence of lysis of *Listeria* bacteria, preferably *L. monocytogenes*, or lysis activity of phage P825 against the *Listeria* bacteria, preferably *L. monocytogenes*, contained in the sample, wherein the presence of lysis of *Listeria* bacteria, preferably *L. monocytogenes*, or lysis activity of a phage of the invention, preferably. P825, against *Listeria* bacteria, preferably *L. monocytogenes*, is indicative of the presence of *Listeria* bacteria, preferably *L. monocytogenes*. In various embodiments, the sample of (i) is obtained from a food product, a food processing equipment, a food storage container, or a patient suspected of suffering from a bacterial contamination comprising *Listeria* bacteria, preferably *L. monocytogenes*.

Further Characteristics of PlyP825

The pH optimum for the lytic activity of endolysin PlyP825 was determined (Example 5 and FIG. **5**). PlyP825 exhibit highest lytic activity at neutral to slightly alkaline (basic) pH. Thus, endolysin PlyP825 has a pH optimum at neutral to slightly alkaline (basic) pH. In various embodiments, the endolysin provided by the present invention is characterized as having a pH optimum at about pH 8.5 with respect to its lytic activity. In various other embodiments, the endolysin provided by the present invention is characterized as exhibiting improved lytic activity at a pH of about 5.5, about 6.5 or about 7.5.

The salt optimum (NaCl) for the lytic activity of endolysin PlyP825 was determined (Example 6 and FIG. **6**). PlyP825 exhibits highest lytic activities in the concentration range of about 150-250 mM NaCl. Thus, endolysin PlyP825 has a salt (NaCl) optimum of about 150-250 mM NaCl. In various embodiments, the endolysin provided by the present invention is characterized as having a salt (NaCl) optimum at a concentration in the range of about 150-250 mM NaCl. In various other embodiments, the endolysin provided by the present invention is characterized as having a salt (NaCl) optimum at a concentration of any one of about 150 mM, about 200 mM or 250 mM NaCl.

The lytic activity of endolysin PlyP825 in the presence of different concentrations of EDTA was determined (Example 7 and FIG. **7**). While Ply511 was inactivated already at a concentration of 1 mM EDTA, the relative lytic activity of PlyP825 remained at a level of about 70% up to a concentration of about 25 mM EDTA.

The minimum inhibitory concentration (MIC) of endolysin PlyP825 against *Listeria monocytogenes* ProCC S1095 sv 1/2a, *Listeria monocytogenes* ProCC S1135 sv 3a, *Listeria monocytogenes* ProCC S776 sv 4b, and *Listeria* innocua ProCC S1147 sv 6a was determined (Example 8 and FIG. **8**). The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent at which the visible growth of a microorganism is suppressed (Andrews et al. 2001). The MIC values varied depending on the *Listeria* strain tested. For inhibiting *Listeria monocytogenes* ProCC S1095 sv 1/2a and *Listeria monocytogenes* ProCC S1135 sv 3a less PlyP825 protein was required than for inhibiting *Listeria monocytogenes* ProCC S776 sv 4b. The MIC values for PlyP825 are, depending on the *Listeria* strain tested, about 2.3-times lower, or up to 17.75-times higher than the MIC values for PlyP40.

Listeria Serovar 3 Specific Bacteriophage

The present invention provides bacteriophage capable of lysing *Listeria* serovar 3 obtainable by (a) plating a sample containing bacteriophage and *Listeria* bacteria serovar 3 using agar plates to obtain plaques, and (b) purifying the phage contained within the one or more plaques obtained.

In various embodiments, the step of plating a phage-containing sample and *Listeria* bacteria serovar 3 comprises mixing a phage-containing sample and *Listeria* serovar 3 host cells in molten, "soft" agar. The resulting suspension is then poured on to an appropriate "nutrient" basal agar medium to form a thin "top layer" which hardens and immobilises the bacteria. In various embodiments, the step of plating a phage-containing sample and *Listeria* bacteria serovar 3 follows the double agar layer method as described by Adams (1959).

During incubation the uninfected *Listeria* bacteria multiply to form a confluent lawn of bacterial growth over the surface of the plate. Each infected bacterium bursts after a short time and liberates progeny phages that infect adjacent bacteria, which in turn are lysed. This "chain" reaction spreads in a circular motion until brought to a halt by a decline in bacterial metabolism. Plaques are zones of bacterial lysis caused by bacteriophage action and appear as circular zones of lysis on lawns of bacterial cells.

Phages may be purified by removing, picking off, a well isolated plaque using either a Pasteur pipette or more crudely, but just as effectively, a wire loop. Using a sterile Pasteur pipette, the area around the plaque is stabbed and pieces of soft area are "sucked" into the pipette. The agar should be gently broken into smaller pieces with the wire-loop, mixed briefly with a vortex-mixer and left for 5-10 minutes at ambient temperature. The phage suspension may then be filter-sterilised through a 0.45 mμ syringe-mounted, filtration unit to remove any bacteria including phage-resistant host bacteria.

In various embodiments, the sample is an environmental sample, preferably a sample from environmental water, more preferably a water sample from a rivulet. In various embodiments, the sample is a phage suspension.

In case of the phage-containing sample being a phage suspension, the step of plating a phage-containing sample and *Listeria* bacteria serovar 3 comprises mixing a small volume of a dilution of a phage suspension and *Listeria* serovar 3 host cells in molten, "soft" agar.

FURTHER DEFINITIONS

In the present invention, "Percentage (%) of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

The terms "identical" or percent "identity", in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or sub-sequences that are the same or have a specified percentage of amino acid resides or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical". This definition also refers to the complement of a test sequence. Optionally, the identity exists over a region that is at least about 50 amino acids or nucleotides in length, or more preferably over a region that is 75-100 amino acids or nucleotides in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

The terms nucleic acid molecule and nucleic acid sequence may be used herein interchangeably.

As discussed herein there are numerous variants of the proteins and polypeptides of the present invention. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within protein molecules according to the present invention. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known to the ones skilled in the art. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one amino acid residue has been removed and a different amino acid residue inserted in its place such that a conservative substitution is obtained. The meaning of a conservative substitution is well known to the person skilled in the art.

Certain post-translational modifications are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains, acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl. Such post-translational modifications are also contemplated by the present invention.

The term "*Listeria*" as used herein means the bacterial genus *Listeria*. In the present invention, the genus *Listeria* encompasses all known *Listeria* species. In particular, in the present invention the genus *Listeria* includes, but is not limited to, the following *Listeria* species: *L. monocytogenes, L. seeligeri, L. ivanovii, L. innocua, L. welshimeri, L. grayi* ssp. *grayi*, and *L. grayi* ssp. *murrayi.*

In the present invention, the preferred *Listeria* species is a *Listeria* species that is pathogenic to human beings and/or animals.

In various embodiments of the present invention, the preferred *Listeria* species is *Listeria monocytogenes*, which is pathogen to both human and animals. This applies in particular to the therapeutic and non-therapeutic methods of the present invention.

In the present invention, *Listeria* serovars 1/2, 3, and 4 include, but are not limited to, *Listeria monocytogenes* serovars 1/2, 3, and 4, respectively.

In various embodiments, the preferred *Listeria monocytogenes* serovar is serovar 1/2. In various other embodiments, the preferred *Listeria monocytogenes* serovar is sero-

var 3. In various further embodiments, the preferred *Listeria monocytogenes* serovar is serovar 4.

In the present invention *Listeria monocytogenes* includes serotypes 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7. In various embodiments, the *Listeria* species is selected from the group consisting of *L. monocytogenes* serotype 1/2a, *L. monocytogenes* serotype 1/2b, *L. monocytogenes* serotype 1/2c, *L. monocytogenes* serotype 3a, *L. monocytogenes* serotype 3b, *L. monocytogenes* serotype 3c, *L. monocytogenes* serotype 4a, *L. monocytogenes* serotype 4ab, *L. monocytogenes* serotype 4b, *L. monocytogenes* serotype 4c, *L. monocytogenes* serotype 4d, *L. monocytogenes* serotype 4e, and *L. monocytogenes* serotype 7.

In more preferred embodiments of the present invention the *Listeria* species is selected from the group consisting of *L. monocytogenes* 1142 serovar 1/2a, *L. monocytogenes* 1042 serovar 4b, *L. monocytogenes* 1019 serovar 4c, *L. monocytogenes* 1001 serovar 1/2c, *L. monocytogenes* EGDe serovar 1/2a, *L. monocytogenes* SLCC 7150 serovar 1/2a, *L. monocytogenes* SLCC 7154 serovar 1/2c, *L. monocytogenes* SLCC 7290 serovar 1/2c, *L. monocytogenes* 0756062 serovar 1/2c, *L. monocytogenes* WSLC1485 serovar 1/3a, *L. monocytogenes* WSLC 11082 serovar 1/3c, *L. monocytogenes* WSLC 11083 serovar 1/3c, *L. monocytogenes* ScottA serovar 4b, *L. monocytogenes* WSLC 1048 serovar 4d, *L. monocytogenes* 8309032 serovar 4d, and *L. monocytogenes* 8309033 serovar 4e.

In various embodiments, the preferred *Listeria* species is *Listeria* ivanovii, which is pathogenic to animals. In preferred embodiments, the *Listeria* species is *Listeria* ivanovii serotype 5.

The literature discloses reports about diseases in human beings resulting from infection with *Listeria seeligeri* (Rocourt et al. 1987) and *L. ivanovii* (Cummins et al. 1994). In the present invention *Listeria seeligeri* includes serotypes I/2a, I/2b, I/2c, 4b, 4c, 4d, and 6b. In various embodiments, the *Listeria* species is selected from the group consisting of *L. seeligeri* serotype I/2a, serotype I/2b, serotype I/2c, serotype 4b, serotype 4c, serotype 4d, and serotype 6b.

In the present invention *Listeria* innocua includes serotypes 3, 6a, 6b, 4ab, and U/S. In various embodiments, the *Listeria* species is selected from the group consisting of *L. innocua* serotype 3, *L. innocua* serotype 6a, *L. innocua* serotype 6b, *L. innocua* serotype 4ab, and *L. innocua* serotype U/S. Preferably, *L. innocua* is *L. innocua* 2011 serotype 6a.

In the present invention *Listeria welshimeri* includes serotypes 1/2a, 4c, 6a, 6b, and U/S. In various embodiments, the *Listeria* species is selected from the group consisting of *L. welshimeri* serotype 1/2a, *L. welshimeri* serotype 4c, *L. welshimeri* serotype 6a, *L. welshimeri* serotype 6b, and *L. welshimeri* serotype U/S.

In the present invention *Listeria grayi* includes serotype *Grayi*. In various embodiments, the *Listeria* species is *L. grayi* serotype *Grayi*.

In the present invention, the terms "serotype" and "serovar" may be used interchangeably.

In the present invention, the terms "controlling *Listeria* contamination" and "controlling undesired *Listeria* colonization" may be used interchangeably.

The term "endolysin", as used herein, denotes enzymes that are naturally encoded by bacteriophages and are produced by them at the end of their life cycle in the host to lyse the host cell and thereby release the progeny phages. As described in the background section, endolysins are comprised of at least one enzymatically active domain (EAD) and a non-enzymatically active cell binding domain (CBD). The EADs can exhibit different enzymatic activities as described herein, such as, e.g., N-acetyl-muramoyl-L-alanin amidase, (endo)-peptidase, transglycosylase, glycosyl hydrolase, (N-acetyl)-muramidase, or N-acetyl-glucosaminidase. The terms "endolysin(s)" and "lysin(s)" may be used herein interchangeably.

The term "domain" or "protein domain", as used herein, denotes a portion of an amino acid sequence that either has a specific functional and/or structural property. On the basis of amino acid sequence homologies, domains can frequently be predicted by employing appropriate computer programs that compare the amino acid sequences in freely available databases with known domains, e.g., Conserved Domain Database (CDD) at the NCBI (Marchler-Bauer et al., 2005, Nucleic Acids Res. 33, D192-6), Pfam (Finn et al., 2006, Nucleic Acids Research 34, D247-D251), or SMART (Schultz et al., 1998, Proc. Natl. Acad. Sci. USA 95, 5857-5864, Letunic et al., 2006, Nucleic Acids Res 34, D257-D260).

Whenever reference is made to the activity of the polypeptide of SEQ ID NO: 2 (PlyP825), the endolysin activity of PlyP825 is meant. Specifically, the endolysin activity of the polypeptide of SEQ ID NO: 2 (PlyP825) is the lytic activity of the polypeptide of SEQ ID NO: 2 (PlyP825) against *Listeria* bacterial cells described herein, preferably against pathogenic *Listeria* bacterial cells, more preferably *Listeria monocytogenes*. In general, the enzymatic activity of the endolysin of SEQ ID NO: 2 is analogous to the enzymatic activity of known endolysins exhibiting lytic activity against *Listeria* bacterial cells. More specifically, the lytic activity of the endolysin PlyP825 is hydrolytic activity, still more specifically hydrolytic activity against peptidoglycan in the cell wall of *Listeria* bacterial cells. Therefore, the lytic activity of the endolysin PlyP825 may also be described as peptidoglycan hydrolase activity.

As described herein, the EAD of SEQ ID NO: 4 has lytic activity against *Listeria* bacterial cells. In particular, the lytic activity of the EAD of SEQ ID NO: 4 is defined as lytic activity against *Listeria* bacterial cells. More specifically, the enzymatic activity of the EAD of SEQ ID NO: 4 is analogous to the enzymatic activity of known EADs exhibiting lytic activity against *Listeria* bacterial cells. Given the fact that the polypeptide of SEQ ID NO: 4 represents the EAD of the endolysin of SEQ ID NO: 2, and given that EADs from *Listeria* bacteriophages are known and described in the art, the nature of the lytic activity of the EAD of SEQ ID NO: 4 of the present invention is clear to the skilled person. In various embodiments of the present invention, the lytic activity of the EAD of SEQ ID NO: 4 against *Listeria* bacterial cells is peptidoglycan hydrolase activity, i.e. hydrolytic activity against peptidoglycan in the cell wall of *Listeria* bacterial cells. The peptidoglycan hydrolase activity of the EAD of SEQ ID NO: 4 may also be called peptidoglycan-digesting activity or muralytic activity. In various embodiments, the lytic activity of the EAD of SEQ ID NO: 4 is muramidase activity or N-Acteyl-glucosaminidase activity. In various embodiments, the lytic activity of the EAD of SEQ ID NO: 4 is amidase activity or endopeptidase activity. Preferably, the lytic activity of the EAD of SEQ ID NO: 4 is peptidoglycan amidase activity. More preferably, the lytic activity of the EAD of SEQ ID NO: 4 is L-muramoyl-L-alanine amidase activity, D-alanyl-glycyl endopeptidase activity, or D-6-meso-DAP-peptidase or meso-DAP-D-Ala peptidase activity. In various embodiments, the lytic activity of the EAD of SEQ ID NO: 4 is peptidoglycan transglycosylase activity. More preferably, the lytic activity of the EAD of SEQ ID NO: 4 is murein transglycosylase

activity. In various embodiments, the lytic activity of the EAD of SEQ ID NO: 4 is peptidase activity, preferably carboxypeptidase activity. In various embodiments, the lytic activity of the EAD of SEQ ID NO: 4 is glycosyl hydrolase activity. In various embodiments, the lytic activity of the EAD of SEQ ID NO: 4 is N-acetylmuramoyl-L-alanine amidase activity. In various embodiments, the lytic activity of the EAD of SEQ ID NO: 4 is cysteine histidine-dependent amidohydrolase/peptidase activity.

As described herein, the CBD of SEQ ID NO: 6 has cell wall binding activity. This cell wall binding activity provides for targeting the lysin to its substrate, namely the peptidoglycan of *Listeria* bacterial cells. Therefore, in particular the cell wall binding activity of the CBD of SEQ ID NO: 6 is *Listeria* cell wall binding activity. In general, the enzymatic activity of the CBD of SEQ ID NO: 6 is analogous to the enzymatic activity of known CBDs that likewise provide for targeting lysin to its substrate in the cell wall of *Listeria* bacterial cells. Given the fact that the polypeptide of SEQ ID NO: 6 represents the CBD of the endolysin of SEQ ID NO: 2, and given that CBDs from *Listeria* bacteriophages are known and described in the art, the nature of the cell wall binding activity of the EAD of SEQ ID NO: 6 of the present invention is clear to the skilled person. Accordingly, it is also clear to the skilled person that CBDs according to the present invention have no or no significant hydrolytic activity like the EADs, i.e. CBDs according to the present invention have no or no significant hydrolytic activity against *Listeria* bacterial cell walls. Here, no or no significant hydrolytic activity is intended to describe the situation whereby the hydrolytic activity of a CBD of the present invention is not sufficient to prevent the application of such a CBD to bind to the cell wall of a *Listeria* bacterial cell. A CBD according to the present invention is supposed to be a protein, which has no or no significant hydrolytic activity itself.

In various embodiments, the cell wall binding activity of the CBD of SEQ ID NO: 6 is binding to peptidoglycan of the cell wall of *Listeria* bacterial cells. Preferably, the cell wall binding activity of the CBD of SEQ ID NO: 6 is binding to a carbohydrate or cholin moiety in the cell wall of *Listeria* bacterial cells. More preferably, the cell wall binding activity of the CBD of SEQ ID NO: 6 is binding to a carbohydrate of the peptidoglycan or teichoic acid or lipoteichoic acid in the cell wall of *Listeria* bacterial cells.

The terms "protein" and "polypeptide" are used in the present invention interchangeably. As used herein, the term endolysin denotes an enzyme. Accordingly, whenever reference is made herein to a protein or polypeptide of the present invention, this also includes endolysins of the present invention. The terms "endolysin(s)" and "endolysin protein(s)" or "endolysin polypeptide(s)" may be used herein interchangeably.

Furthermore, basically the terms "protein" and "polypeptide" as used herein also encompass any "chimeric lysin" provided by the present invention. However, for clarity reasons concerning the scope of the present invention sometimes reference is made herein to "proteins and polypeptides of the present invention" on the one hand, and "chimeric lysins of the present invention" on the other hand. The terms "chimeric lysin(s)" and "chimeric endolysin" may be used herein interchangeably. Furthermore, the terms "chimeric (endo)lysin(s)" and "chimeric (endo)lysin protein(s)" or "chimeric (endo)lysin polypeptide(s)" may be used herein interchangeably.

Furthermore, the term "sv" represent the well known abbreviation of the term "serovar".

When particular embodiments of the invention are described herein, the corresponding paragraphs/text passages of the description invariably make reference to means and/or methods described elsewhere in the description. In this context, terms like "according to the present invention", "of the present invention" and "provided by the present invention" are used. That is, when a particular embodiment of the invention is described in a certain paragraph or text passage, reference is made to means and/or methods "according to the present invention" or "of the present invention", which are described elsewhere in the description. For a particular embodiment described, such references are intended to incorporate for the particular embodiment all means and/or methods, which are described elsewhere in the description and which are provided by the present invention and therefore form part of the scope of the invention. For example, if the description of a particular embodiment refers to "the endolysin according to the present invention" or "the endolysin of the present invention", or "the endolysin provided by the present invention", it is intended that all endolysins, which are described elsewhere in the description, and which are provided by the present invention and therefore form part of the scope of the invention, are applicable to the particular embodiment. This particularly applies, for example, to fragments and variants of polypeptides according to the present invention, which are defined in the present invention and which are applicable to the various embodiments described throughout the application text.

The above principle applies to all embodiments making use of terms like "according to the present invention", "of the present invention" and "provided by the present invention". It goes without saying that not each embodiment described herein can specifically mention the means and/or methods of the invention, which are already defined elsewhere in the description, and which are applicable to the various embodiments described throughout the application text. Otherwise, each patent application would comprise several hundreds of description pages.

Furthermore, terms like "in various embodiments" and "in various other/further embodiments" mean "in various embodiments of the present invention" and "in various other/further embodiments of the present invention"

The invention is exemplified by the examples, which are not considered to limit the scope of the present invention.

Example 1

Lytic Activity of Phage ProCC P825

The phage P825 provided by the present invention exhibits lytic activity against *Listeria* serovars 1/2, 3, 4, 5 and 6. As demonstrated by the inventors, phage P825 completely inhibited growth of *Listeria monocytogenes* strains.

250 µl overnight culture of different *Listeria* strains were added to 3 ml TB-top-agar (TB-medium, 0.75% (v/v) agar, 2 mM $CaCl_2$, 10 mM $MgSO_4$), and poured into TB-agar plates. 5 µl of phage P825 (10^9 pfu/ml) were spotted onto the top-agar plates and dried for about 30 minutes. The plates were incubated overnight at room temperature. Evaluation of lysis spots demonstrated that phage P825 was lytic for all *Listeria* strains tested. Evaluation of lysis spots was performed as follows:

More than 75% of strains tested from one serovar show a lysis spot: "+"

Less than 25% of strains tested from one serovar show a lysis spot: "−"

Not determined: nn.

TABLE 1

Comparison of lytic activity of *Listeria* phages on *Listeria* serovars ("+", "−" and "nn" in accordance with the above definition)

Listeria phage	Serovar 1/2	Serovar 3	Serovar 4	Serovar 5	Serovar 6
P825	+	+	+	+	+
A511	+	−	+	+	+
P100	+	−	+	+	+
A118	+	−	−	nn	nn
A500	−	−	+	nn	+
P40	−	−	+	+	+
PhiLM4	−	−	+	nn	+

Phage P825 has been shown to be lytic against *Listeria* serovars 1/2, 3, 4, 5 and 6. The host range is broader than that of known phages A511, P100, A118, A500, P40, and PhiLM4, as shown in the above Table 1. Importantly, novel phage P825 is capable of lysing *Listeria* serovar 3, which is one of the clinically relevant *Listeria* serovars. This activity is unique to novel phage P825. Known *Listeria*-specific bacteriophages A511, P100, A118, A500, P40, and PhiLM4 do not share this property.

Furthermore, phage P825 not only inhibited growth but actually reduced *Listeria* titers. As confirmed by enrichment studies, applying phage P825 completely eradicated *Listeria* bacteria. The lysis spectrum of phage P825 has been shown to be consistent with the binding specificity provided by the tailspike protein of phage P825.

Example 2

Proteolytic Stability of PlyP825 Compared to Ply511 and PlyP40

In order to compare the proteolytic sensitivity of the three endolysins they were Trypsin-digested in equimolar amounts. Aliquots were retained and analyzed after 0 and 3 min incubation at room temperature (FIG. **1**). As shown in FIG. **1**, PlyP825 possesses less proteolytic degradation sites compared to Ply511 and PlyP40.

Example 3

Lytic Activity of PlyP825 Against a Broad Range of *Listeria* Serovars

PlyP825 was analyzed for its activity against different *Listeria* strains with serovars 1/2, 3, 4, 5 and 6. Overnight cultures of *Listeria* cells were poured 1:6 in LB-Top Agar in plates. Onto the solidified agar 2 μg of PlyP825 was spotted. After incubation over night at 30° C. all 22 strains tested were lysed by the endolysin PlyP825 (Table 2). Thus, PlyP825 is a broad range *Listeria* endolysin.

TABLE 2

22 *Listeria* strains tested for lysis by PlyP825. ProCC S: Culture Collection Number of Hyglos Invest GmbH, Bernried, Germany. "+" indicates lysis of this strain by PlyP825.

ProCC S	Strain	Serovar	PlyP825
1095	*Listeria monocytogenes* EGDe	1/2a	+
995	*Listeria monocytogenes* SLCC 7150	1/2a	+
1153	*Listeria seeligeri* WSLC 40140	1/2b	+
1002	*Listeria monocytogenes* SLCC 7154	1/2c	+
1003	*Listeria monocytogenes* SLCC 7290	1/2c	+
2867	*Listeria monocytogenes* 0756062	1/2c	+
1135	*Listeria monocytogenes* WSLC1485	3a	+
1154	*Listeria seeligeri* WSLC 40127	3b	+
2991	*Listeria seeligeri* WSLC 41113	3b	+
2974	*Listeria monocytogenes* WSLC 11082	3c	+
2975	*Listeria monocytogenes* WSLC 11083	3c	+
776	*Listeria monocytogenes* ScottA	4b	+
1144	*Listeria monocytogenes* WSLC 1048	4d	+
2919	*Listeria monocytogenes* 8309032	4d	+
2920	*Listeria monocytogenes* 8309033	4e	+
857	*Listeria ivanovii* WSLC 3009	5	+
1014	*Listeria ivanovii* SLCC 4706	5	+
1164	*Listeria ivanovii* ssp. *londoniensis* WSLC 30167	5	+
1150	*Listeria ivanovii* ssp *ivanovii* WSLC 30165	5"	+
1147	*Listeria innocua* WSLC2011	6a	+
773	*Listeria innocua* WSLC 2012	6b	+
1754	*Listeria seeligeri* WSLC 41116	6b	+

Example 4

Minimum Bactericidal Concentration of PlyP825 in Buffer and Milk

The minimum bactericidal concentrations (MBCs) of the endolysins PlyP825, Ply511 and PlyP40 in buffer and in milk were determined and compared.

For determining the MBC in buffer pH 6 the endolysin enzymes were incubated with 10^5 cells/ml of strains *Listeria monocytogenes* EGDe sv 1/2a and *Listeria* innocua WSLC2011 sv 6a in buffer (20 mM sodium phosphate, 50 mM sodium chloride, 0.05% Tween pH 6) at 30° C. After 1 h the samples were plated and cell numbers counted. FIG. **2** shows the results: PlyP825 reduces effectively pathogenic and non-pathogenic *Listeria* cells in buffer: 0.032 μg/ml endolysin were sufficient to reduce 4.5 (WSLC2011) or 3.1 (EGDe) orders of magnitude of *Listeria* cells. This is about 0.5 to 1.5 log more than Ply511 and 0.9-1.3 log more than PlyP40 were able to reduce with the same protein concentration.

For determining the MBC in milk the enzymes were incubated with 10^5 cells/ml of strains *Listeria monocytogenes* EGDe sv 1/2a and *Listeria* innocua WSLC2011 sv 6a in milk with 1.5% fat at 30° C. After 3 h the samples were plated and cell numbers counted. FIG. **3** shows the results: PlyP825 shows the highest *Listeria* cell reduction in milk. Independent from the test strain PlyP825 reduces 1.4-1.7 orders of magnitude more cells than the other two broad *Listeria* endolysins Ply511 and PlyP40 in milk with 1.5% fat. Besides the enzymes were incubated with 10^5 cells/ml of strains *Listeria monocytogenes* EGDe sv 1/2a in milk with 3.5% fat at 30° C. After 3 h the samples were plated and cell numbers counted. FIG. **4** shows the results. Also in milk with 3.5% fat PlyP825 reduces the highest cell number.

Example 5

pH Optimum of PlyP825

The pH optimum for the lytic activity of endolysin PlyP825 was determined and compared with that of endo-

lysins Ply511 and PlyP40. The results are shown in FIG. **5**. The lytic activity as a function of the pH was determined applying photometric lysis tests. In particular, heat-inactivated cells of *Listeria monocytogenes* ProCC S1095 sv 1/2a were suspended in buffer (50 mM sodium citrate, 50 mM NaH_2PO_4, 50 mM borate and 100 mM NaCl), which was adjusted to pH values of 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5, respectively. As shown in FIG. **5**, PlyP825 and Ply511 exhibit highest lytic activity at neutral to slightly alkaline (basic) pH. Thus, endolysins PlyP825 and Ply511 have a pH optimum at neutral to slightly alkaline (basic) pH. The result shown for Ply511 confirms the pH optimum described in the literature (Pieper et al. 2005). PlyP40 exhibits highest lytic activity at acidic pH. Thus, endolysin PlyP40 has a pH optimum at acidic pH.

Example 6

Salt (NaCl) Optimum of PlyP825

The salt optimum (NaCl) for the lytic activity of endolysin PlyP825 was determined and compared with that of endolysins Ply511 and PlyP40. The results are shown in FIG. **6**. The lytic activity of endolysins Ply511, PlyP40 and PlyP825 against *Listeria monocytogenes* ProCC S1095 sv 1/2a was determined at pH6 for concentrations of 0 mM, 10 mM, 50 mM, 100 mM, 150 mM, 250 mM, and 500 mM NaCl. As shown in FIG. **6**, endolysins Ply511 and PlyP825 exhibit highest lytic activities in the concentration range of about 150-250 mM NaCl. Thus, endolysins Ply511 and PlyP825 have a salt (NaCl) optimum of about 150-250 mM NaCl. Furthermore, as shown in FIG. **6** endolysin PlyP40 exhibits highest lytic activity at a concentration of about 150 mM NaCl. Thus, endolysin PlyP40 has a salt (NaCl) optimum of about 150 mM NaCl.

Example 7

Relative Lytic Activity of PlyP825 in the Presence of EDTA

The lytic activity of endolysin PlyP825 in the presence of different concentrations of EDTA was determined and compared with that of endolysins Ply511 and PlyP40. The results are shown in FIG. **7**. The lytic activity as a function of the EDTA concentration was determined applying photometric lysis tests using *Listeria monocytogenes* ProCC S1095 sv 1/2a as reference strain. The incubation period was one hour at pH 6 and different concentrations of EDTA. As shown in FIG. **7**, Ply511 was inactivated already at a concentration of 1 mM EDTA, and at a concentration of 250 mM EDTA the residual activity was about 1%. Furthermore, as shown in FIG. **7**, the relative lytic activity of PlyP40 remained almost unchanged up to a concentration of 100 mM EDTA, and the relative lytic activity of PlyP825 remained at a level of about 70% up to a concentration of about 25 mM EDTA. At a concentration of 250 mM EDTA the residual lytic activity of PlyP825 was about 2.5%.

Example 8

MIC of PlyP825 Against *Listeria*

The minimum inhibitory concentration (MIC) of endolysin PlyP825 against *Listeria monocytogenes* ProCC S1095 sv 1/2a, *Listeria monocytogenes* ProCC S1135 sv 3a, *Listeria monocytogenes* ProCC S776 sv 4b, and *Listeria innocua* ProCC S1147 sv 6a was determined and compared with that of endolysins Ply511 and PlyP40. The results are shown in FIG. **8**. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent at which the visible growth of a microorganism is suppressed (Andrews et al. 2001). For determining the MIC cells of *Listeria monocytogenes* ProCC S1095 sv 1/2a, *Listeria monocytogenes* ProCC S1135 sv 3a, *Listeria monocytogenes* ProCC S776 sv 4b, and *Listeria innocua* ProCC S1147 sv 6a, respectively, were incubated in TB medium at pH 6. The growth of the *Listeria* strains tested was observed by determining optical density (OD). As shown in FIG. **8**, the MIC values varied depending on the *Listeria* strain tested. For inhibiting *Listeria monocytogenes* ProCC S1095 sv 1/2a and *Listeria monocytogenes* ProCC S1135 sv 3a in general less protein was required than for inhibiting *Listeria monocytogenes* ProCC S776 sv 4b and *Listeria innocua* ProCC S1147 sv 6a. Ply511 shows the lowest MIC values 0.10 to 3.34 pmol/ml. The concentrations for PlyP40 were higher than the concentrations for Ply511, namely by a factor of about 1.75 to about 3.5. The MIC values for PlyP825 are, depending on the *Listeria* strain tested, about 2.3-times lower, or up to 17.75-times higher than the MIC values for PlyP40.

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Asp Val Ile Trp Ala Val Asp Ala Lys Phe Lys Lys Ile Val Ala Ala
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Ala Gly Ala Pro Lys His Ala Thr Pro Lys Pro Pro Ala Lys Pro Ala
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Thr Ser Gly Met Tyr Val Tyr Phe Pro Ala Gly Lys Gly Thr Trp Ser
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Val Tyr Pro Leu Asn Lys Ala Pro Val Lys Ala Asn Ala Ile Gly Ala
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Ile Asn Pro Ser Lys Phe Gly Gly Leu Thr Tyr Lys Val Glu Lys Asn
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The invention claimed is:

1. A food product comprising a bacteriophage having a genome

i) comprising the DNA sequence of SEQ ID NO: 7;

ii) having at least 90% or 95% sequence identity with the DNA sequence of SEQ ID NO: 7; or

iii) having at least 90% or 95% sequence identity with the DNA sequence of the genome of bacteriophage ProCC P825 deposited under Accession No. DSM 23783;

wherein the food product is selected from the group consisting of a dairy product, a fruit product, a vegetable product, a meat product, and a fish product.

2. The food product of claim **1**, which is a dairy product.

3. The food product of claim **1**, which is a fruit product.

4. The food product of claim **1**, which is a vegetable product.

5. The food product of claim **1**, which is a meat product.

6. The food product of claim **1**, which is a fish product.

7. The dairy product of claim **2**, which is a pasteurized dairy product.

8. The dairy product of claim **2**, selected from the group consisting of yoghurt, ice cream, cheese and butter.

9. The meat product of claim **5**, selected from the group consisting of pate, hot dog, bologna, salami and cold cuts.

10. The food product of claim **1**, wherein the food product has undergone thermal treatment prior to introducing the bacteriophage.

11. The food product of claim **10**, wherein the thermal treatment is at a temperature of at least 70° C.

12. The food product of claim **2**, wherein the bacteriophage has a genome comprising the DNA sequence of SEQ ID NO: 7.

13. The food product of claim **1**, wherein the bacteriophage has a genome having at least 90% or 95% sequence identity with the DNA sequence of SEQ ID NO: 7.

14. The food product of claim **1**, wherein the bacteriophage has a genome having at least 90% or 95% sequence identity with the DNA sequence of the genome of bacteriophage ProCC P825 deposited under Accession No. DSM 23783.

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